University of Alberta

A Prospective Study of Physiological Hyperarousal and Coping as Correlates of Symptoms of Acute Stress Disorder and Posttraumatic Stress Disorder in Motor Vehicle Crash Survivors

by

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Abstract

The primary purpose of this dissertation research was to identify emerging psychological and contextual variables that contribute to the development of acute stress disorder (ASD) and posttraumatic stress disorder (PTSD) following a motor vehicle crash (MVC) and to explore their linkages to neurobiological tests of autonomic arousal and hypothalamic-pituitary-adrenal (HPA) axis activity. An integrative, prospective, repeated measures, correlational design was used. Meeting criteria for the ASD group was associated with increased symptom frequency and intensity on all PTSD and Impact of Event Scale (IES) subscales. Hyperarousal symptoms were the most frequently experienced of the three symptom clusters that must be present for a diagnosis of ASD or PTSD. High hyperarousal levels were associated with increased anxiety symptoms, peritraumatic dissociation, and use of coping strategies at both two- and six-weeks post-MVC and with lower daytime cortisol levels. High hyperarousal levels two weeks post-MVC were strongly predictive of PTSD at six weeks post-MVC. The expected associations between the sympathetic-adrenal medullary (SAM) and HPA systems and the SAM system and PTSD were not found. Together, the findings underscore the severity of stress-related symptoms that MVCs can evoke in individuals and the complexity of interactions among components of stress response systems.

A secondary repeated measures study assessed acoustic startle reactivity in healthy women during their mid-luteal and mid-follicular menstrual cycles. No significant menstrual phase effects on startle amplitude, habituation, latency, latency facilitation or PPI were found. Women showed significant habituation and greatest PPI at the shortest prepulse interstimulus interval in both phases. These findings were integrated into the discussion of the startle data for the pre-menopausal female participants in the MVC study.

Dedication

This book is lovingly dedicated to my husband, Gerry, who encourages me in all that I do and understands that my quest for knowledge is never-ending.

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Table of Contents

Chapter One: Prologue 1

Introduction 1

Significance 2

Significance for nursing 4

Background 5

Concept of "traumatic event" 5

Acute Stress Disorder and Posttraumatic Stress Disorder 6

Hyperarousal 9

Comorbidity 13

Motor vehicle crash-related trauma 14

Overview of dissertation papers and chapter V 18

Summary 23

References 24

Chapter Two (Paper): An Integrative Science Approach: Value Added in Stress Research 30

Integrative science as a concept 30

Stress as a phenomenon of interest to nursing 32

Cognitive and physiological approaches to understanding stress 33

Cognitive approaches to understanding stress 34

Physiological approaches to understanding stress 36

An integrative understanding of stress and its impact 38

An integrative understanding of stress in nursing practice 40

Implications of an integrative science for nursing research 40

Criticisms of an integrative science for nursing 41

Summary 43

References 44

Chapter Three (Paper): Stress Hormones: How Do They Measure Up? 48

Introduction 48

Neuroendocrine responses to stress 49

Cortisol response to stress 50

Cortisol and implications for health 51

Stress hormone measurement 54

Challenge studies 55

Methods and issues in cortisol measurement 57

Advantages of salivary measurement 61

Factors influencing cortisol levels 63

Summary 68

References 69

Chapter Four (Paper): The influence of Menstrual Phase on Startle

Reactivity in Healthy Women 76

Background 76

Method 79

Participants 79

Startle protocol 80

Study design and data analyses 83

Results 85

Discussion 88

References 92

Chapter Five: A Prospective Study of Physiological Hyperarousal and Coping as Correlates of Symptoms of Acute Stress Disorder and Posttraumatic Stress Disorder in Motor Vehicle Crash Survivors 96
Background 96
Method 98
Hypotheses 98
Design 99
Recruitment protocol 100
Measurement of psychological variables 100
Neurophysiological measures 102
Data analyses 105
Results 107
Sample characteristics 107
Psychosocial outcomes 109
Physiological outcomes 118
Discussion 126
Conclusion 134
References 135
Chapter Six: Postscript 141
Appendix A 145
Appendix B 148
Appendix C 150
Appendix D 151

List of Tables

Page

Table 5.1. Differences in total COPE score and COPE subscale scores by ASD group..... 110

Table 5.2. Differences in PTSD (CAPS-I) symptom clusters by ASD group..... 111

Table 5.3. Dependent variables which showed significance by hyperarousal group..... 115

Table 5.4. Differences in MASQ subscales by hyperarousal group..... 116

Table 5.5. Differences in PTSD (CAPS-I) symptom clusters by hyperarousal group..... 117

Table 5.6. Patterns of salivary cortisol levels by hyperarousal group..... 124

List of Figures

		Page
Figure 3.1	HPA axis activation	52
Figure 3.2	Circadian pattern of cortisol release	60
Figure 4.1	Relationship between menstrual phase on startle amplitude and habituation	86
Figure 4.2	Relationship between menstrual phases and startle latency	87
Figure 4.3.	Relationship between menstrual phase and prepulse inhibition	88
Figure 5.1.	Relationship between ASD at baseline and PTSD symptom frequency and intensity	112
Figure 5.2.	Relationship between peritraumatic dissociation (ASDI) at Baseline and PTSD symptom severity	113
Figure 5.3.	Relationship between ASD and startle amplitude and habituation at session 1	119
Figure 5.4	Relationship between PTSD and startle amplitude and habituation at session 2	120
Figure 5.5	Relationship between hyperarousal and startle amplitude and habituation at sessions 1 and 2	121
Figure 5.6	Relationship between ASD and startle amplitude for prepulse trials at all lead intervals (ISI) at session 2	122
Figure 5.7	Relationship between PTSD and startle amplitude for prepulse trials at all lead intervals (ISI) at session 2	122

Figure 5.8 Relationship between hyperarousal and startle amplitude for prepulse trials at all lead intervals (ISI) and for pulse alone (no prepulse) trials at session 2 123

Figure 5.9 Relationship between hyperarousal group and salivary cortisol levels at baseline and pre- and post-startle at sessions 1 and 2 125

Figure 6.1 Startle amplitude and habituation in MP females as compared to MVC females 143

CHAPTER ONE

Prologue

Introduction

This mixed-format[1] dissertation is the culmination of a comprehensive doctoral program of education and research that focused on the psychoneuroendocrinology of traumatic stress. The overall aim of my research work was to identify emerging psychological and contextual variables that contribute to the development of Acute Stress Disorder (ASD) and Posttraumatic Stress Disorder (PTSD) following a specific traumatic event and to explore their linkages to neurobiological tests of autonomic arousal and hypothalamic-pituitary-adrenal (HPA) axis activity. This doctoral research comprised two research studies and supportive coursework that led to two published manuscripts (Chapters 2 & 3), one manuscript in review (Chapter 4), and one chapter (5) yet to be developed into manuscripts for publication.

My interest in psychological and physiological stress responses to traumatic events emerged very early in my nursing career, when I cared for clients and their families in emergency departments and intensive care units. I observed the suffering that highly stressful events can evoke. As a result of this experience, my Master's thesis focused on the study of psychological stress and coping in spouses of conscious and unconscious patients on life support subsequent to an unexpected traumatic event, such as a motor vehicle crash (MVC), work-related accident or unexpected surgical outcome (King, 1988; King & Gregor, 1985). From this research it became clear that if we are to be better able to help meet the needs of individuals who are coping with highly stressful events, a more comprehensive understanding of not only the range of coping strategies used but of the psychological and biological consequences of their use is needed.

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[1] A mixed format dissertation is a blending of published and as yet unpublished research.

Significance

During the past two decades, evidence of the potentially deleterious effects of traumatic stress on psychological and physiological health has been accumulating. While the majority of research has focused on chronic traumatic stress, under the rubric of PTSD, acute traumatic stress, under the rubric ASD, has been gaining research interest. Despite this, traumatic stress disorders continue to represent an important challenge to health care professions and there remains much to learn about these distressing disorders (Shalev, 2001)

Epidemiological studies indicate that traumatic stress disorders are a major health problem worldwide (Brunello et al., 2001; Davidson, 2001). The National Comorbidity Survey that was conducted in 48 states in the United States in 1995 found that rates of exposure to one or more traumatic events were 51.2% in women and 60.6% in men (Meltzer-Brody, Hidalgo, Conner, & Davidson, 2000). Although actual numbers vary according to type of trauma experienced and other factors, it has been estimated that about 10 to 20% of people exposed to some type of severe trauma will develop PTSD (Brunello et al.; Yehuda, 2002). The highest incidence of PTSD has been reported in victims of interpersonal trauma (defined as physical and/or sexual abuse) (Yehuda). PTSD is considered to be among the most common of psychiatric disorders and affects all segments of the population. The lifetime prevalence of PTSD in the general population is thought to be in the range of 8% to 12%, with women being twice as likely as men to experience PTSD (Breslau, 2001; Bursztajn, Joshi, Sutherland, & Tomb, 1999; Tolin & Foa, 2006). Yehuda (2002) suggested that the higher prevalence of PTSD in women may be due, at least in part, to the increased frequency with which women experience interpersonal trauma.

In addition to being one of the most common psychiatric disorders, PTSD often causes significant impairment in quality of life and can persist for many years. PTSD is characterized by distressing symptoms and re-experiencing of the event as though it were recurring for months, years, or a lifetime. Even those who recover from prolonged PTSD are likely to have some residual symptoms and a degree of dysfunction (Shalev, 2001; Vieweg, Julius, Fernandez, Beatty-Brooks, Hettema, & Pandurangi, 2006). They may also be particularly vulnerable to subsequent stress and may develop the full PTSD syndrome again if they are once more exposed to significant trauma, a process known as reactivation (Shalev).

PTSD is a significant public health issue. Individuals with PTSD reportedly use the health care system more frequently than do individuals who have not been exposed to trauma. Both the direct costs associated with increased use of the health care system and the indirect costs associated with decreased productivity at work and impaired social relationships contribute to the significant cost of PTSD to society (Meltzer-Brody et al., 2000). High rates of self-medication with alcohol and other addictive substances subsequent to the development of PTSD contribute to its burden to the individual, family, and society (Bursztajn et al., 1999). Yet, PTSD has been described as "remarkably underdiagnosed" and undertreated, with no single type of management being consistently effective, "probably because the underlying pathophysiology of PTSD is not completely understood" (Bursztajn et al.). Improved early identification of those at risk for developing PTSD and increased psychobiological understanding of traumatic stress symptomatology may eventually lead to more effective attenuating and restorative interventions for individuals with ASD and PTSD. Current clinical guidelines suggest that while many individuals are resilient and recover without treatment, some trauma survivors may benefit from cognitive-behavioral therapy (CBT) or pharmacotherapy. CBT

includes techniques such as cognitive restructuring, exposure therapy, and eye movement desensitization and reprocessing. CBT may help to reduce trauma symptoms and prevent the emergence of chronic PTSD (Ballenger et al, 2004). Antiadrenergics early post-trauma may also help to prevent development of PTSD (Boehnlein & Kinzie, 2007). Selective serotonin reuptake inhibitors (SSRIs) are first line medications for most symptoms of PTSD, although antidepressants, atypical antipsychotics and other agents may be used (Baldwin et al., 2005; Ballenger, Davidson, Lecrubier, Nutt, Marshall, Nemeroff, Shalev, & Yehuda, 2004).

Significance for Nursing

Nurses are concerned about individuals' experiences with stress and the potential for these experiences to impact health and well-being. Although most people adapt, for some individuals traumatic experiences continue to reverberate and cause considerable distress and disruption of daily living for years after the event (Rachman, 2001). Despite enduring distressing or even tormenting symptoms, many people with ASD and PTSD do not come forward for professional help and may subsequently be at greater risk for long-term health problems (Ballenger, 2000). Attempting to reduce vulnerability to these adverse effects is a critical health issue. Given the high incidence of exposure to traumatic events in the general population, nurses are likely to come into contact with traumatized individuals in any practice arena. If nurses are to systematically identify and help people with ASD and PTSD to manage distressing symptoms, such as those of hyperarousal (hypervigilance, irritability, sleep disturbances, increased startle, increased heart rate), and promote adaptation, a better understanding of the neurophysiological basis of the symptoms and the adaptive processes they use is needed. Multidimensional assessment of the effects of traumatic stress is essential for evidence-based, informed nursing practice.

Background

In this section, literature that addresses diagnoses and symptoms of acute and posttraumatic stress disorders in general is first reviewed. Hyperarousal, as a concept central to the primary dissertation study, is discussed in more detail. Hyperarousal, acute and posttraumatic stress disorders, and coping are then discussed with specific reference to studies of MVC survivors, since these studies provide contextual information that informed my primary dissertation study.

Concept of "Traumatic Event"

According to the DSM-IV, a traumatic event is one in which 'the person experienced, witnessed or was confronted with an event which involved actual or threatened death or serious injury or a threat to the physical integrity of self or others' and to which the person responded with 'horror or helplessness' (American Psychiatric Association, 1994). McFarlane (2000) suggested that it is the quality and nature of the emotional memories that traumatic events generate that differentiates them from other types of life events. Traumatic events can be naturally occurring, human-caused, or interpersonal.

Experiences of traumatic events can lead to a variety of outcomes. Although the majority of people who are exposed to traumatic life events adapt, a significant proportion may develop psychological and/or physiological stress-related health problems. Examples of some health problems that have been linked to stressful life events are: cardiovascular disease, gastrointestinal disorders, cancer, autoimmune disorders, alcohol abuse, depression, and other anxiety disorders (Baum & Posluszny, 1999; Campbell et al., 2002; Carney, McMahon, Freedland, Becker, & Krantz, 1998; Ehring, Ehlers, & Glucksman, 2006; Sephton, Sapolsky, Kraemer, & Spiegel, 2000).

Acute Stress Disorder and Posttraumatic Stress Disorder

Both ASD and PTSD are categorized as anxiety disorders in the fourth edition of the *Diagnostic and Statistical Manual of Mental Disorders* (DSM-IV). Both ASD and PTSD are characterized by the coexistence of three symptom clusters. These symptom clusters are re-experiencing, avoidance, and hyperarousal. What differentiates the two conditions is the time period in which symptoms emerge following the traumatic event, the duration of symptoms, and prevalence of symptoms of dissociation (Yehuda & Wong, 2000). In ASD, symptoms must last for at least two days and occur within four weeks of the traumatic event. In PTSD, the symptoms must last for at least one month and emerge from one month to several months or even years after the traumatic event (Yehuda & Wong). Symptoms of dissociation (derealization, reduced awareness, numbing, detachment, and dissociative amnesia) are a diagnostic difference between ASD and PTSD, in that dissociation is a distinct criterion for the diagnosis of ASD but is only inferred with PTSD (American Psychiatric Association, 1994). This shared symptom profile and the frequent development of PTSD subsequent to ASD have produced controversy about whether ASD is a discrete mental disorder or early PTSD (Kangas, Henry, Bryant, 2005; Marshall, Spitzer, & Liebowitz, 1999).

When 'reexperiencing', traumatized individuals often relive the event in their minds through flashbacks, vivid imagery, and nightmares, despite not wanting to do so. They may feel very distressed and have physical symptoms such as sweating, tachycardia, and tachypnea in response to intrusive reminders of the event (Ballenger, 2000; Shalev, 2001; Yehuda & Wong, 2000). Avoidance can be manifested by attempts to avoid objects or situations that may elicit reminders of the event, forgetting details about the event, or feelings of emotional numbness or disconnectedness from others. Hyperarousal may manifest in a range of physiological symptoms, such as sleep

disturbances, inability to concentrate, irritability, anger, hypervigilance (feeling alert and on guard and scanning the environment for signs of threat), and exaggerated startle response (Yehuda & Wong).

In addition to symptoms of reexperiencing, avoidance, and hyperarousal, in order to meet DSM-IV criteria for ASD individuals must also experience three of the following dissociative symptoms: depersonalization, derealization, reduced awareness, numbing, detachment, and dissociative amnesia (American Psychiatric Association, 1994; Harvey & Bryant, 1999c). An individual experiencing dissociation may be unable to recall an important aspect of the trauma or feel emotionally unresponsive (Yehuda & Wong, 2000). Some symptoms of dissociation, such as numbing and impaired recall of aspects of the trauma event, may overlap symptoms of avoidance. There is some discrepancy in the literature as to whether dissociation is a coping strategy or a manifestation of pathology. Functional magnetic resonance imaging studies have provided evidence of a pathological process in dissociation (Lanius et al., 2005). Those who argue that dissociation is a coping strategy view it as a way of protecting the individual from becoming overwhelmed with the traumatic event, thereby helping to maintain psychological integrity (Harvey & Bryant, 1999c; Medina, Mejia, Schell, Dawson, & Margolon, 1999). Despite this protective intent, the use of dissociation as a coping strategy has been associated with increased risk of PTSD (Classen et al., 1998). One reason for this may be that dissociation impairs the emotional processing that is needed for effective coping (Rachman, 2001).

Although several researchers found that dissociative reactions are associated with more severe and prolonged stress responses and increased risk for PTSD (Classen et al., 1998; Grieger et al., 2000; Shalev, Peri, Canetti, & Schreiber, 1996; Yehuda & Wong, 2000), others found that dissociation does not add predictive power for the

development of PTSD to the overall diagnosis of ASD (Brewin, Andrews, Rose, & Kirk, 1999; Harvey & Bryant, 1999c). Brewin et al., in a prospective study of 157 victims of violent assaults, showed that ASD and high levels of reexperiencing/arousal symptoms were predictive of later development of PTSD. Harvey and Bryant (1999b) found that in a study of 92 survivors of MVCs, 13% met criteria for ASD and 21% for subsyndromal ASD. Of the subsyndromal group, 79% satisfied criteria for all clusters except dissociation. The implication of this is that a significant proportion of acutely traumatized individuals may suffer marked distress without meeting full criteria for ASD and potentially may not receive early intervention when needed (Harvey & Bryant, 1999c). They suggested that research is needed to assess the extent to which individuals who meet all criteria for ASD except dissociation are at risk of developing PTSD. In the same study, they found that 75% of people experience hyperarousal symptoms in the first month following a MVC and suggested that future research could usefully investigate the role of hyperarousal symptoms in relation to trauma adaptation. The research reported in this dissertation addresses both these areas of needed investigation.

The impact of traumatic events may be reflected in changes in cortisol levels. Levels of cortisol, as the final output of the HPA axis, rise in response to an acute stressor in healthy individuals without PTSD (Yehuda, 2001). However, cortisol levels reported in studies of people in the immediate aftermath of trauma suggest that cortisol levels can be low in response to an extremely traumatic experience, before criteria for a diagnosis of PTSD are met (McFarlane, Atchison, & Yehuda,1997; Yehuda, 2006). One explanation that has been suggested for this is that sensitization to glucocorticoid activity after repeated or extreme stress could result in upregulation of the number of glucocorticoid receptors, with increased negative feedback; this increased feedback decreases cortisol secretion and may lead to chronic hypocortisolism (Boyer 2000).

Changes in affinity and density of various receptors involved in stress hormone systems may contribute to abnormal cortisol levels (Yehuda, 2001). Low cortisol levels in PTSD may be a vulnerability marker with increased incidence in the offspring of parents with PTSD (Yehuda, Bierer, Schmeidler, Aferiat, Breslau, & Dolan, 2000). Alternatively, other factors that influence cortisol responses in traumatic events have yet to be identified.

Evidence is accumulating that a diagnosis of ASD is highly predictive of the later development of PTSD. In a retrospective study of factory workers, Grieger et al. (2000) found there were no cases of PTSD in workers who did not first meet criteria for ASD. A prospective study of MVC survivors found that approximately 80% of people who initially have ASD meet criteria for PTSD six months later (Bryant, 1999).

McFarlane (2000) suggested, based on a literature review, that whether or not PTSD emerges in response to a traumatic event "depends on the ability of the individual to modify the associated hyperarousal and neurobiological cascade" (p. 19). Since hyperarousal is an important concept in traumatic stress and is a focus of this dissertation, it is discussed in more detail in the following section.

Hyperarousal

Physiological hyperarousal is the most frequently experienced of the three symptom clusters in ASD or PTSD. Harvey and Bryant (1999b) found that 75% of people experience hyperarousal symptoms in the first month following a MVC. Hyperarousal is commonly manifested as irritability, anger, difficulty in concentrating, disturbed sleep, hypervigilance, increased heart rate, and exaggerated startle (Brunello et al., 2001; Bursztajn et al., 1999; Mathew, Coplan, & Gorman, 2001). There is evidence that hyperarousal in the acute post trauma phase is associated with long-term PTSD (Harvey & Bryant, 1999a; McFarlane, 2000; Shalev et al., 1998 Marshall, Schell, Glynn, & Shetty,

2006). Hyperarousal symptoms have also been associated with increased alcohol and drug use (Jacobsen, Southwick, & Kosten, 2001; Shalev, 2001).

While hyperarousal implies alertness and readiness for action or information processing, actual information processing may be inferior during states of high arousal (Coupland, 2000). Whereas increased autonomic activity and arousal are thought to serve the important adaptive function of mobilizing resources in response to actual threat, excessive and inappropriate physiological arousal has been associated with symptoms of anxiety and stress disorders that involve perceived threat (Gencoz, Gencoz, & Joiner, 2000; Bonnet & Arand, 1996). Hyperarousal may impede cognitive processing that is needed for accurate appraisals of a stressful situation and effective coping choices (McFarlane, 2000; Shalev, 2001).

Several researchers suggested that corticotrophin releasing hormone (CRH) hyperactivity may underlie the physiologic hyperarousal associated with PTSD and other stress disorders (Heim et al., 2000; Koob, 1999; Nutt, 2000; Pacak, 2000). Serotonin may indirectly contribute to hyperarousal by enhancing CRH secretion (Cassano & D'mello, 2001; Nutt). During prolonged or uncontrollable stress, CRH increases norepinephrine turnover in specific brain regions, including the locus ceruleus, hypothalamus, hippocampus, amygdala, and cerebral cortex and activates the sympathetic division of the autonomic nervous system. As well, CRH activates the HPA system by stimulating the pituitary to release ACTH. Elevated levels of CRH in the brain, particularly in the amygdala, potentiate fear-related behavioral responses, including the startle response, and may mediate both the symptoms of hyperarousal and the increased risk for substance abuse and dependence in PTSD (Jacobsen et al., 2001).

Cortisol may modulate the startle reflex. Buchanan, Brechtel, Sollers, and Lovallo (2001) used 20 mg of hydrocortisone to test the effects of cortisol on the non-modulated

and emotion-modulated acoustic startle reflex in a small sample of 12 healthy men and women (aged 20-40 yrs). During non-modulated startle, oral hydrocortisone had a dose effect, with 5 mg increasing and 20 mg decreasing eyeblink reflex magnitude compared to placebo. During emotion-modulation with affectively valenced (positive and negative) slides, 20 mg of hydrocortisone reduced eyeblink reflex magnitude. These findings suggest that cortisol may exert dose-dependent effects on the startle pathway (Buchanan et al.). They also provide evidence supporting linkages between the HPA and sympathetic-adrenal-medullary (SAM) stress response systems.

Exaggerated startle, as a component of the hyperarousal symptom cluster, has been utilized as an experimental paradigm in PTSD. The startle eyeblink response is viewed as a robust and reliable component of the startle reflex and is commonly used as an index of peripheral and central autonomic reactivity (Filion, Dawson, & Schell, 1998; Metzger, Orr, Berry, & Ahern, 1999; Morgan, 1997, Shalev et al., 2000). Although the startle reflex is a protective response that develops in early infancy in healthy humans, abnormalities of the startle reflex have been associated with anxiety and stress-related disorders (Larsen, 2001). Examples of abnormalities of the startle reflex include a startle response that is of greater amplitude than normal and decreased prepulse inhibition (PPI). PPI occurs when a stimulus that is preceded by a weaker pre-stimulus results in a decrease in startle amplitude. Increased startle reactivity and particularly decreased PPI have been associated with decreased ability to filter out extraneous environmental stimuli in people with schizophrenia (Braff & Geyer, 1990). It is unknown whether decreased ability to filter out extraneous environmental stimuli may be related to increased startle reactivity and hyperarousal in PTSD, since no reports were found that included PPI assessment in startle studies in PTSD. Studies of PTSD have focused more closely on the physiological reactivity to unconditioned stimuli, such as the

exaggerated startle response to sudden or loud noises (Medina et al., 1999; Guthrie & Bryant, 2005). Pacak (2000) found preliminary evidence of distinctive patterns of neuroendocrine responses to different stressors in animal studies that suggest there may be stressor-specific differences in the activation of the HPA and SAM systems. As result of these findings, I assessed physiological indicators of unconditioned SAM reactivity to acoustic stimuli and HPA activity in people exposed to a single type of traumatic event (i.e., a MVC).

Although the majority of studies, including that of Shalev et al. (2000), report sensitization of physiological reactivity (startle and heart rate) as PTSD progresses, other studies suggest that PTSD that arises from chronic stressors may be related to suppression rather than sensitization of physiological reactivity. Medina et al. (1999) found, in a study of 52 women with PTSD related to partner aggression, that PTSD symptom severity was significantly associated with startle eyeblink magnitude. However, contrary to expectation, they found a negative correlation between PTSD symptom severity scores and the magnitude of startle eyeblink responses. They suggested that this finding could be explained on the basis of perceived situation and coping, in that when a situation is perceived as inescapable, individuals may use different coping responses, such as dissociation. Suppression of autonomic physiological responses has also been reported in rape survivors with high levels of dissociation (Griffin, Resick, & Mechanic, 1997). Medina et al. (1999) suggested that it may be important to distinguish between aversive situations which occur only once, are limited in duration, and offer opportunity for some control and use of active coping strategies, and aversive situations of repeated victimization or prolonged duration in which the individual feels helpless and possible coping choices are limited. However, coping was not actually assessed in the study. They also suggested that further investigation of potential psychological and

physiological differences among different subgroups of traumatized individuals is needed (Medina et al.).

Although evidence of a robust circadian pattern of acoustic startle was reported in rats (Chabot & Taylor, 1992; Frankland & Ralph, 1995), it is not known whether there may be a circadian rhythm for startle responses in humans. For this reason, I assessed startle at the same time of day for both sessions for each subject. Subjects were assessed either in early morning or late afternoon sessions to enable factor control for time of day. No studies that measured both cortisol levels and startle responsivity to examine the effects of MVC-related stress or PTSD were found. However, exogenous corticosteroid was found to influence startle in a study of healthy volunteers (Buchanan et al., 2001).

These studies had implications for the design of my dissertation studies. An acute stressor that is entirely unexpected, such as a MVC, may result in different startle and coping patterns than chronic stressors that are characterized by ongoing fearful anticipation, such as repeated interpersonal trauma. Studies of chronic stressors in PTSD populations do little to inform our understanding of the relationships between emerging acute stress symptoms and psychological and biological variables that increase risk (Classen et al., 1998). Prospective studies commenced in the immediate aftermath of a traumatic event are needed to accomplish this goal. In my primary dissertation study, I examined prospectively and longitudinally the relationships between psychological variables, coping, emerging trauma-related symptoms, and measures of autonomic arousal and HPA axis activity in MVC survivors.

Comorbidity

PTSD has high rates of comorbidity with substance abuse and dependence (23%), alcohol dependence (75%), personality disorder (20%), and major depression

(20%), (Khouzam & Donnelly, 2001). Others have found higher rates of comorbid depression in PTSD (e.g., 40%: Shalev et al., 1998). Other comorbid conditions include panic disorder, social phobia, agoraphobia, generalized anxiety disorder, and bipolar disorder (Khouzam & Donnelly). Also, PTSD is more strongly associated with suicidal behavior than any other anxiety disorder (Ballenger, 2000).

Maes, Mylle, Delmeire, and Altamura (2000) found that crash victims with PTSD and comorbid major depression or any other anxiety disorder suffer more clinical distress and present with more severe symptoms of PTSD than victims with PTSD alone. The extent of physical injury, female gender, younger age, loss of control, type of trauma, and the degree of exposure to the traumatic event were found to determine the occurrence of these comorbid disorders (Maes et al.). Chronic pain syndromes, such as whiplash-associated disorders and fibromyalgia, may also develop along with PTSD in MVC survivors (McLean, Clauw, Abelson, & Liberzon, 2005; Palyo & Beck, 2005).

Physiological arousal may be useful in discriminating between anxiety and depression. Hyperarousal has been associated with anxiety symptoms and right parietotemporal region activation; in contrast, low levels of arousal have been associated with anhedonia, depressive symptoms and inhibition of the right parietotemporal region (Gencoz et al., 2000). Given the comorbidity of depression and PTSD, measures of hyperarousal have potential to provide important discriminatory information in PTSD studies, including my MVC study. However, differences in arousal in PTSD when comorbid depression is and is not present have yet to be determined.

Motor Vehicle Crash-Related Trauma

MVCs are among the most frequent traumatic events culminating in PTSD (Butler & Moffic, 1999). Although the responses of individuals to stressful events are highly variable, increased stress has been demonstrated to be associated with certain

conditions that may be present in a MVC event. These include exposure to traumatic events with little or no time for anticipatory preparation (Jacobson, 1983), exposure to ambiguous or uncertain events (Lazarus & Launier, 1978; Mishel, 1984), exposure to events that provide tangible reminders (King, 1988) and exposure to events which involve an important aspect of life, such as health, injury or survival (Blanchard, Hickling, Taylor, Loos, Forneris, & Jaccard, 1996; Cagnetta & Cicognani, 1999; Maes et al., 2000; Schnyder, Moergeli, Klaghofer, & Buddeberg, 2001). MVC survivors may experience any or all of these conditions. MVCs are unexpected. In addition to physical injury, MVCs may also involve injury to loved ones and damage to property. When crashes are due to human error, emotional reactions may include anger and blaming others or guilt, shame and self-blame (Butler & Moffic). Immediate reactions and symptoms following a MVC may predict development of PTSD (Frommberger, Stieglitz, Nyberg, Schlickewei, Kuner, & Berger, 1998).

Mayou, Bryant, and Ehlers (2001) found that while the majority of MVC survivors (n=773) reported symptom improvement between the 3 and 12 month assessments, one-third reported phobic travel anxiety, general anxiety, and depression at both times. Phobic travel anxiety was eight times higher in motorcyclists. Male and female passengers did worse than drivers. A hypothesized reason for the latter finding is that lack of control over events on the road may make passengers feel more threatened. Persistent health problems, rumination about the crash, and negative interpretations of intrusive recollections at 3 months were strong predictors of PTSD at one year (Mayou et al.).

Given the high prevalence and the potential for detrimental health effects in MVC survivors, surprisingly few studies of this population have included physiological measures of stress. However, those that have yielded information that is useful as a

basis for further research. Bryant, Harvey, Guthrie, and Moulds (2000) used heart rate and blood pressure (BP) to assess arousal in 146 non-brain injured MVC survivors. Hyperarousal at one month posttrauma, as indicated by increased heart rate and motor restlessness, was predictive of PTSD at six months. Elevated BP was not predictive of PTSD. Harvey and Bryant (2000) assessed the same parameters in 79 people with mild brain injury. Hyperarousal findings were the same as in the non-brain injured sample assessed by Bryant et al. (2000). While all symptoms of ASD were predictive of PTSD, the symptoms with the strongest positive predictive power were numbing, reduced awareness, depersonalization, recurrent images/thoughts, nightmares, relieving the traumatic experience, avoidance, and motor restlessness (Harvey & Bryant).

Studies of coping in trauma contexts are typically based on coping inventories (Brom, Kleber, & Hofman, 1993; Bryant & Harvey, 1995; Carver, 1998; Gil & Caspi, 2006; Jeavons, 2000; Schnyder et al., 2001). These studies reported a predominant use of emotion-focused and avoidance coping strategies posttrauma. Longitudinal studies found that more active coping strategies tend to be used over time (Brom et al.; Jeavons).

In a longitudinal prospective study of coping in MVC survivors (n=72), Jeavons (2000) found a significant decrease in trauma symptoms over a six month period posttrauma and a strong positive relationship between initial emotion-focused and avoidance coping styles and later trauma scores. Emotion-focused and avoidance coping scores did not change significantly over time and were related to higher trauma scores at six months. There was a significant increase in task-oriented coping over time but this was not related to trauma scores at six months. Early coping scores predicted later trauma scores (Jeavons). These results are interesting as they indicate that the emotional impact of the event is persistent and that those with stronger emotional

responses and who use avoidance as a coping strategy initially may be more vulnerable to later trauma symptoms. These findings, if confirmed in further studies, support the importance of finding ways to help reduce the emotional impact of the event in this population.

Although avoidance is one of the symptom clusters for PTSD, it may also be a coping strategy. Traumatized individuals commonly avoid situations that lead to fear and anxiety (Ledoux & Gorman, 2001). For example, driving phobias, limitations on driving, and anxious behavior as passengers are avoidance symptoms as well as coping strategies to avoid reexperiencing the crash and emotions associated with it or being involved in yet another crash (Butler & Moffic, 1999). Although talking through the trauma until it becomes less frightening may be therapeutic for some, it may thwart coping by avoidance in others. Recent findings about debriefing after trauma suggest that debriefing may not be helpful and perhaps even harmful to some, especially if imposed on them (Ballenger, 2000; Bursztajn et al., 1999).

There is evidence that people may begin to self-medicate with alcohol or other drugs as an avoidance coping strategy directed to reduction of symptoms of generalized autonomic hyperarousal, anxiety, fear and phobias subsequent to trauma (Bursztajn et al., 1999). Research indicates that PTSD develops first and alcohol or drug addiction arises secondarily (Bursztajn et al.). King (1988) found that tangible reminders of a traumatic event limited the use of denial as a coping strategy. In MVC survivors, injuries that result in scars, changes in mobility or persistent pain or damage to property may act as tangible reminders and decrease ability to cope by denial or avoidance. The extent of injury suffered may also affect coping (Blanchard & Hickling, 1997)

In summary, there is considerable evidence that MVCs are traumatizing for many people. Variations across studies in the strength of physiological hyperarousal as a

predictor of PTSD may be related to differences in measurement methods used or may reflect stressor-specific activation. There is early evidence that emotion-focused strategies are used most frequently to cope, type of coping strategies used posttrauma may help to predict development of PTSD, and information about coping can assist with the interpretation of trauma symptom severity data. The need is clear for research regarding coping, emerging psychological symptoms and physiological stress responses, including autonomic arousal, in MVC survivors.

Overview of Dissertation Papers & Chapter V

My doctoral research work builds and expands on my Master's work, integrating physiological stress measures with psychological measures to contribute to a better understanding of stress responses to traumatic events. The first two papers are foundational works. In the first paper, I delineate the philosophical value of an integrative science approach to stress research. This paper set the stage for the second paper in which I elaborate on stress measurement, in particular measurement of the hypothalamic-pituitary-adrenal (HPA) axis stress system. In the course of my work on these first two papers, it became apparent that the majority of studies of stress were cross-sectional in design and focused on measuring one of the two physiological stress systems (HPA or SAM). I became interested in discovering how these two stress systems might interrelate with one another, as well as with psychological and behavioral stress responses in trauma-exposed subjects over time. Because motor vehicle crashes are among the most common stressful events contributing to ASD and PTSD in the general adult population, this population was selected for study. Acoustic startle was selected for measurement of the SAM stress system and salivary cortisol was selected for measurement of the HPA axis as these physiological measures are non-invasive and have produced reliable results. An examination of the research literature revealed that

although menstrual phase might influence physiological stress measures, few studies that assessed acoustic startle controlled for this factor. This made interpretation of studies that included females difficult and produced potential confounding effects for my primary study that included women who experienced a MVC. For this reason, I conducted a concurrent study to examine potential menstrual phase effects on acoustic startle in healthy women. The results of this study are presented in the third paper (Chapter Four). The results of my study of MVC survivors are presented in Chapter Five. An overview of each of these papers is presented in the next section.

Paper One - An integrative science approach: Value added in stress research

Integrative approaches to understanding complex health issues can transcend disciplinary boundaries and provide opportunities to view phenomena from diverse perspectives. In this paper I assert that these broad approaches to understanding phenomena of interest to nursing may provide new directions for nursing research and are requisite for delivering safe, responsible, and holistic nursing care. In support of this position, the advantages of integrating evidence derived from physiological and cognitive studies to increase understanding of the phenomenon of stress and stress-related health problems and ultimately develop targeted interventions are explicated. In this paper I describe what is meant by 'integrative science' and why an integrative approach to understanding stress is relevant for nursing practice. Salient differences between two major stress research traditions, cognitive and physiological, are discussed in terms of their respective contributions to our understanding of stress and its impact on health. Specific examples of stress-related health problems are incorporated for illustrative purposes. Broader implications of an integrative approach for nursing practice and research are explored. Finally, I argue that the potential of an integrative approach to

contribute to improvements in human health and well-being outweigh historical biases that have been associated with an integrative science approach.

Paper Two - Stress Hormone Measurement

In this paper some of the biological mechanisms involved in stress responses, the varied approaches used to investigate those responses, and issues in stress hormone measurement are discussed. Stress as a stimulus is integral to dynamic homeostatic functioning. However, evidence of its potentially deleterious effects on health is mounting. The impetus to understand the mechanisms that underlie stress-related negative health outcomes and to prevent the development of stress-related disorders has never been greater. Symptom severity and subjective levels of stress, although frequently assessed in studies of stress in nursing research, may not provide adequate data to fully understand the pervasive effects of chronic or overwhelming stress associated with stress disorders. Measurement of neuroendocrine hormones, such as cortisol, can help to identify bodily changes that are stressor-specific and people at risk for development of stress-related disorders, as well as to evaluate efficacy of interventions aimed at stress reduction. Cortisol, as the peripheral output of one of the major stress response systems, possesses several properties that make its measurement highly useful for investigations of stress. However, the method of collection, the timing of collection and the analyses method all have implications for data interpretation. The biological mechanisms involved in the stress response, why cortisol is commonly measured, and issues and approaches in cortisol measurement are discussed.

Paper Three - The Influence of Menstrual Phase on Startle Reactivity in Healthy Women

In this paper I report my investigation of whether normal fluctuations of female sex hormones across the menstrual phase may be sufficient to influence the startle eyeblink reflex in twenty healthy women. Although abnormalities of the startle reflex have been associated with stress-related disorders that affect both genders, potential differences in startle responses across phases of the menstrual cycle in women have yet to be determined. Estrogens, progesterone and progesterone metabolites have all been shown to exert potent neuromodulatory effects in the CNS. Thus, neurobiological changes that occur during the normal menstrual cycle potentially may influence the startle response. I used a repeated measures design to assess amplitude, latency, and PPI of the acoustic startle eyeblink reflex in women during their follicular and luteal menstrual phases. Effects were assessed within-subjects (women across both phases) and between-groups (follicular and luteal phase women). No significant menstrual phase effects on startle amplitude, habituation, latency, latency facilitation or PPI were found. Women showed significant habituation and greatest PPI at the shortest prepulse interstimulus interval in both phases. Menstrual phase at first test session was related to perceived stress but not to reactivity to startle probes in either follicular or luteal phases. These findings were considered when interpreting the startle data for the pre-menopausal female participants in my motor vehicle crash study.

Chapter 5 - A Prospective Study of Physiological Hyperarousal and Coping as Correlates of Symptoms of Acute Stress Disorder and Posttraumatic Stress Disorder in Motor Vehicle Crash Survivors

In this chapter I report on my primary research study. The purpose of this study was to explore emerging psychological and contextual variables that contribute to the development of ASD and PTSD following a specific traumatic event and their linkages to

neurobiological tests of autonomic arousal and HPA axis activity. Specifically, I used an integrative, multidimensional prospective approach to assess whether physiological hyperarousal (as measured by hyperarousal symptoms, heart rate, and startle responsivity) in the immediate aftermath of a specific traumatic event (a MVC) is associated with the subsequent development of PTSD. I assessed the contribution that choice of coping strategies may make to hyperarousal symptom severity and the development of ASD and PTSD. In addition, I examined the potential relationship between the SAM stress system, as measured by acoustic startle and heart rate, and the HPA system, as measured by salivary cortisol levels. This study is innovative in that it included measurement of both salivary cortisol levels and acoustic startle responses in the same subjects.

Of the 26 MVC survivors in my study, the majority were sufficiently traumatized by the MVC event to be included in the ASD group. Of the ASD group, two-thirds subsequently developed PTSD. Although injuries sustained in the MVCs were non-life threatening, the crash event at the time of occurrence was almost always appraised as a threat to life. Meeting criteria for ASD was associated with increased symptom frequency and intensity on all PTSD and Impact of Event Scale (IES) subscales.

Hyperarousal symptoms were the most frequently experienced of the three symptom clusters that must be present for a diagnosis of ASD or PTSD. High hyperarousal levels were associated with increased anxiety symptoms, peritraumatic dissociation, and use of coping strategies at both two and six weeks post-MVC. High hyperarousal levels two weeks post-MVC were strongly predictive of PTSD at six weeks post-MVC. The expected associations between the SAM and HPA systems and the SAM system and PTSD were not found. Together, the findings of my study underscore

the severity of stress-related symptoms that MVCs can evoke in individuals and the complexity of the interactivity of the SAM and HPA neuroendocrine pathways.

Summary

Traumatic events can shatter individuals' views of the world as a safe place and can cause considerable distress for many years after the event. Potentially, they can exert long-lasting effects on psychological and physical health. Attempting to attenuate these adverse effects is a critical health issue. If emotional and physical health and caring are central to nursing, then nursing needs to play a role in uncovering the health consequences of traumatic events and in the subsequent testing and development of interventions. An integrative, multidimensional research approach may lead to a more complete understanding of the psychological and biological correlates that contribute to the development of stress-related health problems, such as ASD and PTSD, and ultimately lead to development of more effective interventions. MVCs are among the most frequent traumatic events culminating in PTSD. Yet, relatively few prospective studies in MVC survivors have included physiological measures of stress. By examining the emergence of stress disorder symptoms and their neurobiological, psychological, and coping correlates over time, this thesis has contributed to development of this foundational knowledge.

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CHAPTER TWO

An Integrative Science approach: Value Added in Stress Research[1]

In a paper published in 1999, Lorraine Walker posed the question, "Is integrative science necessary for the responsible practice of health professions?" In response, we assert that for some health problems an integrative science perspective makes important contributions to knowledge needed to guide professional nursing practice. In support of this position, the advantages of integrating evidence derived from physiological and cognitive studies to increase understanding of the phenomenon of stress and stress-related health problems and ultimately develop targeted interventions will be explicated. The paper will initially focus on what is meant by 'integrative science' and why an integrated approach to understanding stress is relevant for nursing practice. Salient differences between two major stress research traditions, cognitive and physiological, will then be discussed in terms of their respective contributions to our understanding of stress and its impact on health. Finally, broader implications of an integrative approach for nursing practice and research are explored. Although a comprehensive discussion of all stress theories, measurement strategies, and stress-related health problems is beyond the scope of this article, specific examples are incorporated for illustrative purposes.

Integrative Science as a Concept

Walker (1999) provides a comprehensive discussion of the various ways that 'integrative science' is conceptualized in the literature. These range from the open-ended sharing and juxtaposing of knowledge relevant to solving a problem or

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[1] *A version of this chapter has been published.*
King, S. L., & Hegadoren, K. M. (2006). An integrative science approach: Value added in stress research. Nursing and Health Sciences, 8, 114-119.

studying a phenomenon of interest across disciplines to the development of interdisciplinary models and theories. For the purposes of this paper, integrative science will be considered to transcend disciplinary, paradigm, and methodological boundaries as explicated by Walker:

> By integrative science, I mean those intellectual strategies that scientists employ to span disciplinary or knowledge boundaries so as to more fully study and understand human phenomena. Such science is undertaken not for its own sake, but because advances in knowledge about phenomena of interest are best achieved by stepping across existing knowledge boundaries. (p. 94)
>
> At the very least, an integrative science perspective supports carefully considering the diverse knowledge about a population or a phenomenon to select the most relevant knowledge regardless of where it resides. As a result inquiry undertaken from an integrative science perspective is not committed to disciplinary boundaries, but, rather, draws on relevant knowledge and research methods wherever these may lie. (p.98)

Stress is a phenomenon that lends itself well to an integrative science approach. Stress is universally experienced by human beings and can affect all aspects of human experience, including food intake and digestion, exercise, learning, motivation, attention, work, mood, sleep, and social relationships. Despite its universality, there is not unanimity as to the definition of stress. If the focus is more on the response, then stress can be viewed as an evolution-based arousal state that is essential for survival. Others focus more on stress as a stimulus that taxes or exceeds the adaptive capacity of the individual, resulting in psychological and biological changes that increase risk of health problems (Cohen, Kessler, & Gordon, 1997). However, there is wide acknowledgment that the type of stressor (operationally defined as a stressful stimulus), how it is

perceived by the individual and the duration of exposure all contribute to the impact on health.

Stress as it relates to illness has been studied by a variety of disciplines with differing research traditions. Two research traditions, cognitive and physiological, will be discussed in detail as an example of two traditions that contribute importantly but differently to our understanding of the relationship between stress and illness. Discussion will include the importance of integrating knowledge of stress derived through research by multiple disciplines for a more complete and holistic understanding of stress and its effects on human health and well-being. As Walker noted:

> Interrelated phenomena are often treated as intellectually separate because of specialization boundaries or because of paradigmatic or metaphysical boundaries. This means that the client as an integrated whole is not well served by the way the science is ordered... If the problem is intellectual separation of knowledge relevant to understanding persons or populations, then integrative science as a solution to this problem must be defined by an open-ended sharing and juxtaposing of knowledge relevant to solving that problem. (p. 97)

If we accept that the primary concern of nurses is human health and well-being, that the recipient of nursing care is an "integrated whole", and that the rightful approach to care is holistic, then nurses must necessarily adopt an integrative approach to understanding such phenomena as stress.

Stress as a Phenomenon of Interest to Nursing

Nurses have long been concerned about individuals' responses to stressors. Interactions that nurses have with individuals, whether they occur within the context of administering a medication, explaining a procedure, applying a warm blanket, changing a dressing, or listening to concerns and fears, influence internal physiological stress

responses, as well as overt behavioral responses. Such activities increase or decrease individuals' perceived stress levels, with corresponding biochemical changes within multiple organ systems. Interventions that are perceived as 'stressful" will produce characteristic changes in primary stress response systems. For example, when a nurse approaches a conscious patient bearing equipment to establish an intravenous line, levels of cortisol and epinephrine will increase in the systemic circulation. When cortisol levels surge, the immune system is suppressed and serum glucose levels rise. When epinephrine levels surge, heart rate, blood pressure and subjective anxiety increase. These are normal time-limited responses to a stressor. However, if the individual perceives the stressor as overwhelming or of protracted duration, it may lead to stress-related health problems (Biondi & Picardi, 1999; Chrousos & Gold, 1992). Acute stress experiences may also exacerbate existing health problems (Cohen & Herbert, 1996).

Preventing or attenuating the potentially adverse effects of stress on health is a critical challenge. Nurses have a role to play in systematically identifying people who are at increased risk of stress-related health problems and helping them to avoid or reduce the potentially negative consequences. They also have a role to play in providing targeted interventions to address acute symptom management as well as life style issues with people who have developed stress-related health problems. If nurses are to fulfill these roles, an integrative understanding of stress and its relationship to health is essential.

Cognitive and Physiological Approaches to Understanding Stress

Cognitive and physiological frameworks and research traditions have contributed much to our understanding of stress and how stress influences health and disease processes. Yet they differ in the ways that they conceptualize and measure stress and conceptualize the pathways by which stress leads to health problems. Whereas

cognitive frameworks guide research about the roles of cognitive processes and psychological schemas in individuals' responses to stressful events, physiological frameworks guide studies that increase understanding of the biochemical and biophysical basis of stress-induced symptoms (Baum, 1999). Both research approaches make important contributions to knowledge needed to develop and evaluate interventions that address the potentially negative psychological and physiological consequences of stress.

Cognitive Approaches to Understanding Stress

Cognitive approaches to understanding stress focus primarily on the role of emotional responses, appraisal, and coping strategies in determining vulnerability to health problems (Cohen et al., 1997). Emotional responses can be categorized in terms of the types, duration, and intensity of emotions that are experienced, the valence (e.g., positive or negative) of emotions, and levels of emotional arousal (Stone, 1997). Appraisal processes reflect individuals' perceptions of the meaning and significance of a stressful or potentially stressful situation (Monroe & Kelley, 1997). These perceptions reflect both the historical and current context of an individual's life. Coping processes involve individuals' choices of strategies to reduce or manage their emotional responses to a stressful situation and/or resolve the stressful situation (Lazarus, 1974; Lazarus, 1999).

A large number of methods and instruments have been developed to measure cognitive dimensions of stress responses to naturally occurring and artificially created stressors. Methods commonly used include a range of checklist inventories, structured and semi-structured interview guides, unstructured interviews, self-rating instruments, interviewer-rated instruments, and other paper and pencil tests. Often, combinations of methods and instruments are used to provide more complete information and to control

influencing variables. These methods produce a wealth of useful information about the ways that people appraise, experience, and manage stress in response to acute and chronic stressors that vary in intensity and duration. However, they also pose a number of challenges for both the conduct of research and the interpretation of results. Firstly, measurements of the cognitive processes associated with stress are dependent on individuals' subjective interpretations, reports and evaluations of stressful experiences. As such, they are subject to recall and response biases and other limitations that are commonly associated with subjective measures. Secondly, aspects of cognitive processes, such as mood and coping strategies, are dynamic and changeable (Oakland & Ostell, 1996; Stone, 1997). As a result, retest measurements of cognitive processes are subject to variability and ongoing discussion regarding whether various instruments measure "state" or "trait" elements of behavior. Another challenge is that numerous and diverse extraneous factors can make significant contribution to observed variance among individuals or groups. For example, interpersonal interactions, news events, caffeine intake, time of day or year, personality variables, comorbid psychiatric disorders, and mood are just some of the factors that may potentially influence stress responses. Although optimal control of all potentially influential extraneous variables may not be possible, factors that are most likely to exert the greatest influence on the concept under study can often be identified from the literature and controlled in the research design.

The primary pathway that links stress to illness from the cognitive perspective is that an appraisal of having inadequate resources to manage the current stress load can lead to hopelessness and helplessness, as well as to poor health practices, such as increased smoking, alcohol or substance use, poor diet, lack of exercise, reduced compliance with health care regimes, and disturbed sleep (Cohen et al., 1997). In addition, perceived stress may cause individuals to misinterpret symptoms, perhaps

mistakenly attributing stress symptoms to illness or illness symptoms to stress. Subsequently, health-seeking behaviors in response to symptom experiences and adherence to treatment regimes may be negatively affected (Cohen et al.).

In summary, cognitive traditions increase our understanding of the roles of thoughts, emotions, appraisal, and coping strategies in relation to stressful events. Although cognitive measures can predict associations between cognitive dimensions of stress, such as appraisal or coping patterns, and health practices, in the absence of physiological measures they do not contribute to a full understanding of causal relationships between stress and illness outcomes (Cohen et al., 1997).

Physiological Approaches to Understanding Stress

Physiological approaches to understanding stress focus on activation of biological stress systems in response to internal stimuli or environmental threat. According to physiological approaches, the primary pathways linking stress to illness involve hormonal, immune and neuronal systems (Cohen et al., 1997). These systems are evolutionarily designed to rapidly activate in response to stressors and to turn off when the threat has passed. If an individual cannot remove himself or herself from a situation that is perceived as stressful or is unable to cope with the situation, the stress response may endure, with altered production of stress-related hormones. Prolonged or repeated activation of hormonal and neuronal stress systems are thought to place individuals at increased risk for a range of health problems (Haddy & Clover, 2001). Current theories on posttraumatic stress disorder (PTSD) conceptualize it as a failure of response systems to switch off (Shalev, 2002).

Physiological approaches help us to understand the neuronal and hormonal changes that occur in both the CNS and the periphery during acute and chronic stress. For example, levels of the stress hormone cortisol can increase up to 10-fold in

response to a stressor (Schimmer & Parker, 1996). This acute increase in cortisol in response to stress is protective in the short-term. However, chronic or extreme increases in cortisol may have potentially detrimental effects on hippocampus neurons (Sapolsky, 2000) and immune function (Evans et al., 1997). It may also contribute to mental health problems, such as depression (Heim et al., 2000; Marti et al., 2001; Southwick et al., 1998) and medical problems, such as insulin resistance, hypercholestrolemia, and hypertriglyceridemia (Gibson et al., 1999).

Numerous approaches to understanding physiological dimensions of stress response systems have been used. They include such measures as: (a) sympathetic tone – e.g. heart rate, blood pressure (Haddy & Clover, 2001) and reactivity to startling stimuli (Braff et al., 2001; Sabatinelli et al., 2001; Vanman et al., 1998; Yeomans & Frankland, 1996); (b) basal and stimulated levels of hormones, neurotransmitters and neuropeptides that are involved in the stress responses and the regulatory control of these responses (e.g. epinephrine, norepinephrine, cortisol, and adrenocorticotropic hormone, corticotropin releasing hormone, arginine vasopressin and oxytocin) (Chrousos & Gold, 1992; Yehuda, 2000); (c) immunological function (Cohen & Herbert, 1996; Fox et al., 1999; Glaser et al., 1999); and (d) differential brain activation using neuroimaging techniques (Bremner, 1998). While these methods provide objective data that help to characterize stress responses and show how specific regions of the brain and neuroendocrine systems may be differentially activated in response to various types and intensities of stressors, interpretation of the data in a clinically meaningful way is difficult without reference to psychological or cognitive processes. For example, evidence that hippocampal neurons are vulnerable to chronic exposure to excessive levels of cortisol (Sapolsky, 2000) is more meaningful when interpreted in the context of the increased incidence of hypercortisolemia and its potential contribution to memory

impairment in people with depression. An integrative approach using physiological markers and specific symptom clusters may also allow meaningful sub-typing of complex heterogeneous disorders like depression. Such sub-typing would aid in developing more targeted interventions.

In summary, our understanding of stress would be incomplete if the contributions of one tradition were considered in isolation from the other. Attempts to understand stress-related health problems from a purely biological tradition would fail to address the perceptual and cognitive capacities and uniqueness of individuals. This could help explain why pure pharmacological treatment of depression lacks efficacy in up to 1/3 of individuals who are treated with an antidepressant. Likewise, attempts to understand stress from cognitive dimensions alone would fail to address biological systems, where stress-induced changes may become manifest in physiological, psychological and immunologic illnesses. Either approach alone will not support the multiple levels of interaction between biological systems and psychological schemas.

An Integrative Understanding of Stress & Its Impact

Integration of physiological and cognitive research traditions is necessary for a more comprehensive understanding of phenomenon, such as stress (Biondi & Picardi, 1999; Engel, 1987; Engelberg, 1995; Fava & Sonino, 2000; Herbert, 1997; Martin, 2002; Revelle, 1995; Ryff & Singer, 2000; Urbach, 1997). From an integrative perspective, stress and its attendant emotional responses are viewed as the result of complex interactions of genetic, physiological, cognitive, behavioral, and environmental factors that affect the body's ability to maintain homeostasis and to resist or overcome disease (Ryff & Singer). Stress responses, regulated by neuronal, immune and endocrine systems and heavily influenced by cognitive processes, appear to have important implications for health and illness outcomes. Stress has been shown to influence

memory (Lupien et al.,1997), immune system function (Glaser et al., 1999) and the development of cardiovascular disease (Carney et al., 1998), cancer (Andersen et al., 1994), major depression (Bremner et al., 2000) and other illnesses. PTSD, the quintessential stress-related illness, was first thought to be a rare condition primarily related to war experiences. However, it is now recognized as a relatively common response to events such as motor vehicle collisions and sexual or physical abuse and common in emergency-related professions, such as police, firefighters and ambulance workers. PTSD carries with it significant risk for a protracted longitudinal course and significant comorbidity. Stress has also been shown to influence health-enhancing and health-risk behaviors, including diet, exercise, tobacco and alcohol use, preventive health practices, risk-taking behaviors, interpretation and experience of symptoms, and adherence to medication and treatment regimes (Baum, 1999; Epel et al., 2001; Rabkin & Streuning, 1976; Steptoe et al.,1996). With so many implications for human health and well-being, stress is an important phenomenon in nursing practice and research.

Research approaches from an integrative perspective attempt to measure dimensions of both cognitive and physiological processes to understand the relationships among them and how they may together contribute to stress-related health outcomes. For example, Segal et al. (1996) described a model that integrates neurobiology of affective illness with cognitive science in an effort to understand how individuals experiencing affective disorders, such as major depression, may become increasingly vulnerable to recurrence in the presence of increasingly minimal environmental cues. This kindling and sensitization model describes how patterns of information-processing and neurobiological activity may co-develop and intersect to contribute to this increasing vulnerability to relapse. Research based on such a model may lead to innovative, multidimensional interventions.

An Integrative Understanding of Stress in Nursing Practice

Stress studies using research tools from both cognitive and biological methods can more easily be transferred into clinical practice and health policy. In a reciprocal fashion, many stress-related questions may emerge in nursing practice that an integrative approach may help to answer. For example, surgical nurses may need to proactively recognize the kinds of biochemical changes (e.g., changes in glucose utilization, vascular tone, electrolyte balance, or reactions to pre-op medications) that are associated with appraisals of psychological stress in preoperative patients awaiting surgery (e.g., Lewis et al., 1980). Occupational health nurses may be interested in supporting coping strategies that can attenuate acute stress responses in employees threatened with impending layoffs (e.g., Arnetz et al., 1991). Nurses involved in women's health need to understand the impact of oral contraceptives on physiological and psychological responses to stress (e.g., Kirschbaum et al., 1995). Other questions may emerge from practice for which answers are not yet available and research from an integrative perspective is needed.

Implications of an Integrative Science for Nursing Research

Nursing has shifted away from an empiricist paradigm toward an interpretivist human science paradigm, which focuses on holistic understanding of the experiences of unique human beings. While there are many positive aspects to this movement, the important fact that human beings are embodied beings with biological as well as psychosocial processes must not be overlooked. Jordan (1994 & 1999) suggests that the apparent marginalization of biological sciences in nursing may be the result of nursing efforts to acquire professional autonomy and concerns that science may be detrimental to humanism. These efforts may be misguided. McCrone (1996) cautions that "by neglecting the bio in biopsychosocial" at a time when the neurobiological basis

of stress and mental health problems is becoming increasingly understood, nurses may have insufficient knowledge to provide truly holistic care, particularly for the seriously ill. A holistic approach to care must encompass the biological as well as the psychological and social aspects of being human. Focusing on interpersonal relationships and lived experiences without adequate concern for physical well-being fails to acknowledge the complexity and integrity of human beings. An integrative approach to research can contribute to our understanding of how people adapt and thrive. It can also guide nursing practices that facilitate optimal health. In addition, as Akinsanya (1987) and Schneider and Flaskerud (1999) have suggested, by engaging in biological research in addition to other kinds of research, nurses may generate knowledge that ultimately enhances professional competence and improves the quality and safety of patient care.

Criticisms of an Integrative Science for Nursing

An integrative approach to understanding phenomena, such as stress, may be criticized for being contrary to specific goals of the nursing discipline and for difficulties that may be associated with its implementation. As Walker (1999) has suggested, an integrated science approach that attempts to develop multidisciplinary theoretical frameworks or promotes sharing of knowledge and engaging in interdisciplinary research may threaten nursing efforts directed toward the development of a unique body of knowledge and threaten nursing's professional identity. Some nursing scholars believe that a unique or distinctive body of knowledge and a unique contribution to health care are important as a means of ensuring the survival of the nursing discipline (Mitchell, 1999; Villarruel et al., 2001). At the same time, nursing claims that human beings in need of care and human health are our primary concerns. If these are our primary values, then efforts to protect our body of knowledge and our discipline should not preempt our aim to understand the totality of human experiences and responses.

Therefore, our efforts should be directed toward utilizing and contributing to knowledge that has the potential to improve health irrespective of the sources of that information. As Mitchell (1997) has correctly observed, the concepts of 'human health', 'lived experience', and 'holism' that are phenomena of concern to nursing are not unique to nursing. Indeed, these beliefs and values are part of the discourse of other health professions, as a well as philosophy and social science fields. It may be that our discipline is more likely to flourish by contributing meaningfully to multidimensional research and in working collaboratively with other disciplines. Certainly, there is strong evidence that the major North American research funding agencies favor the development of such research teams.

Engleberg (1995) claimed that, "science demands of investigators that they not only make discoveries, but that they make these discoveries ... a part of the total fabric of science". It follows that access and contributions to knowledge that extends beyond disciplinary realms is appropriate for all professions. The reality is that nursing has always drawn from knowledge of other professions. Zeller (1999) suggested that the unique perspective that nursing has to offer is reflected in our focus on deriving research questions from nursing practice and our goal of utilizing research findings to influence nursing practice. The utility of such an arrangement is obvious. However, if research is strictly utilitarian in serving the questions and needs of nursing practice, new insights, innovations and possibilities that alternative perspectives might provide may be missed. In addition, nursing may miss opportunities to contribute to "the total fabric of science". An integrative approach that transcends disciplinary and knowledge boundaries and provides opportunities to view phenomena from diverse perspectives has the potential to lead to new insights and improved care.

Summary

In summary, complex health problems and phenomena can be better understood from an integrative science perspective. Stress and its relationship to illness is one example of a phenomenon that can be understood more fully from an integrative perspective. An integrated approach to understanding health-related phenomena is imperative for safe, responsible, and holistic nursing practice and for the ongoing evolution of nurses' research agenda.

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CHAPTER THREE

Stress Hormones: How Do They Measure Up?[1]

As evidence of the potentially deleterious effects of stress on health accumulates, the impetus to increase our understanding of the biological effects of high levels of chronic or severe activation of the human stress response is intensifying. Although several neuroendocrine products and neurotransmitters are involved in biological responses to stress, this article is focused on the hypothalamic-pituitary-adrenal (HPA) axis and the rationale and approaches for measuring levels of cortisol, which in humans is the final product of this axis. Abnormal cortisol levels are associated with many common health problems. In addition to providing important information about HPA axis function, cortisol levels can help differentiate among stress and illness experiences in terms of adaptive capacity. This information can then be used to guide development of nursing interventions and evaluate their efficacy. Salivary cortisol measurement is emphasized because it is a practical, reliable and non-invasive approach that provides quantitative data regarding biologically active cortisol levels. It has been suggested that the use of saliva testing in the diagnosis and prevention of disease is about to enter a period of explosive growth (Maniga & Golinsky, 2001).

Nurses are concerned about individuals' experiences with stress and with supporting successful management of stress to promote comfort and health. Although there are some variations in the ways "stress" is defined, common among these

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[1] *A version of this chapter has been published.*
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definitions is that stress is a process in which environmental demands tax or exceed individuals' adaptive capacities, contributing to biological and psychological changes that may place them at risk for illness (Cohen, Kessler, & Gordon, 1997). Self-reported feelings of stress and anxiety, although frequently assessed in research studies, might reflect inaccurate self-assessments of bodily state (Vanman, Dawson, & Brennan, 1998). Biological measures, such as cortisol levels, in addition to behavioral and expressive measures can contribute to a more accurate and comprehensive understanding of stress-related health problems and the therapeutic outcomes of targeted pharmacological and non-pharmacological interventions. Since stress is a universal human experience and has important implications for physical and psychological health, multidimensional assessment of its effects is essential for evidence-based, informed nursing practice.

This article will first provide a brief overview of neuroendocrine responses to stress. A brief discussion of approaches to stress measurement will follow. Measurement of cortisol will be the primary focus. Factors that have been shown to influence cortisol levels, and can thus affect how data from experimental studies are interpreted, will also be discussed.

Neuroendocrine Responses to Stress

Two well recognized stress response systems are activated when a stimulus is perceived as a stressor. These are the sympathetic or autonomic response system and the HPA axis. Activation of the sympathetic system occurs within seconds with resultant increases in secretion of epinephrine from the adrenal medulla and norepinephrine from peripheral and central sympathetic neurons. Activation of the HPA axis occurs more slowly within minutes or hours with increases occurring in release of corticotropin-releasing hormone (CRH) from the hypothalamus, which stimulates the

pituitary to release adrenocorticotropin-releasing hormone (ACTH) into the systemic circulation, which in turn stimulates the adrenal cortex to release the glucocorticoid, cortisol (Baum & Grunberg, 1997). Although arginine vasopressin (AVP) has been shown functionally to play a role in the control of ACTH release, the exact mechanism and interdependence of AVP with CRH is not yet known (Oshima et al., 2000). Pacak (2000) found evidence suggesting that patterns of central neurotransmitter release, and thus HPA axis activity, may be stressor-specific.

Although both the sympathetic and HPA stress systems are important to the overall stress response, the sympathetic system is more closely associated with the initiation of the alarm, or "fight - flight" stage. Numerous animal (maternal deprivation, restraint models, elevated plus maze) and human studies using various experimental paradigms support that the HPA system plays an important role in physiological and psychological coping with a stressor and in moderating the effects of stress on health, mood, behavior, and the development of stress related diseases (Breier 1989; Meaney, Aitken, vanBerkel, Bhatnagar, & Sapolsky, 1988). For this reason, the remainder of this article is focused on the HPA axis.

Cortisol Response to Stress

In humans, cortisol is the primary glucocorticoid. Normally, cortisol is synthesized and secreted in the adrenal cortex at the rate of about 10 mg daily in humans (Schimmer & Parker, 1996). Under basal conditions, cortisol interacts mostly with high affinity mineralocorticoid receptors, which are important for normal homeostatic control of metabolic processes and fluid balance. However, when the HPA axis is activated during a stressful experience, cortisol levels can increase at least 10-fold (Schimmer & Parker). At these levels, cortisol interacts with lower affinity glucocorticoid receptors. Through its interaction with glucocorticoid receptors, cortisol

is hypothesized to promote short-term survival in an acute stress event by increasing glucose and oxygen supply to skeletal muscles and the heart to facilitate flight and to the brain to facilitate short-term remembering; suppressing reproductive, immune, and digestive functions to conserve energy; promoting analgesia; and activating the peripheral autonomic system (Sapolsky, 2000b). Increasing levels of cortisol act as a negative feedback signal to suppress further CRH and ACTH release at the level of the hypothalamus and pituitary, respectively (see Figure 3.1). Following cessation of a stressor under laboratory conditions, free unbound cortisol levels tend to increase further for another 15 to 20 minutes before beginning to decrease (Kirschbaum & Hellhammer, 2000).

Cortisol and Implications for Health

Although cortisol activation in response to stress is protective in the short-term, chronic or extreme activation may have long-term negative consequences (Heim, Ehlert, & Hellhammer, 2000a; Sapolsky, 2000a; Southwick, Yehuda, & Wang, 1998). Chronic or extreme activation can lead to changes in HPA axis activity as evidenced by abnormal cortisol levels, which may in turn increase vulnerability to develop health problems. Normalization of the acute cortisol response after the termination of a stressful event protects against the potentially detrimental effects of glucocorticoids on hippocampus neurons, immune function, and mental health. The hippocampus, which is important in memory and cognition, is the brain region with the largest number of glucocorticoid receptors and thus is both an important glucocorticoid feedback site and vulnerable to glucocorticoid-mediated neurotoxicity. Although hippocampus neurons may be able to regrow and memory deficits are usually temporary, prolonged exposure to glucocorticoid excess could result in extensive hippocampus damage and

permanent memory impairment (Boyer, 2000; Lupien et al., 1998; McEwen,1999; Sapolsky, Armanini, Packan, & Tombaugh, 1987).

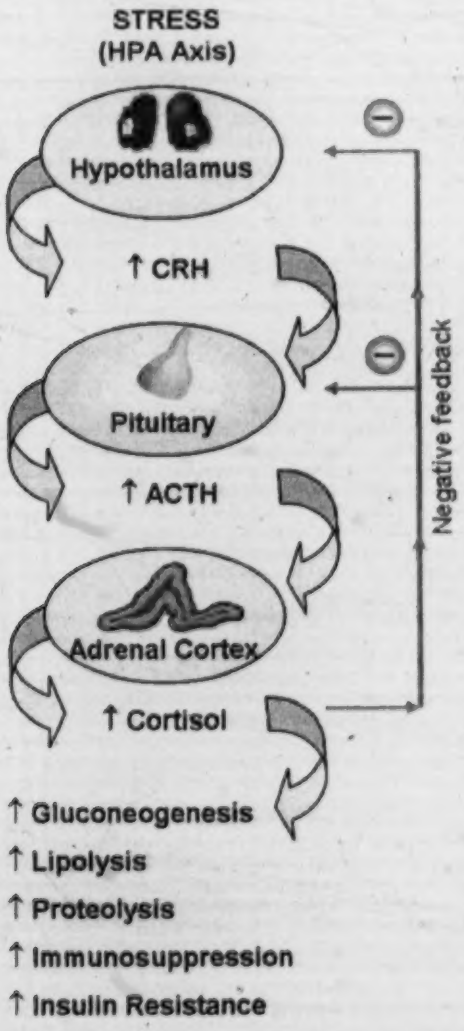

Figure 3.1. Activation of the HPA axis leads to the sequential release of hormones at each level of the axis, culminating in the release of cortisol. Through negative feedback inhibitio n, increasing levels of cortisol suppress further CRH and ACTH release at the level of the hypoth alamus and pituitary.

Cortisol stimulates the immune system and counteracts inflammatory and allergic reactions at normal levels, but can suppress the immune system at excessive levels or when prescribed therapeutically in synthetic drug form (Vanderhaeghe, 2001). Whereas hypercortisolism is thought to be associated with diseases characterized by immunosuppression and reduced ability to fight infections (e.g., Evans & Johnson,1997;Leserman, Petitto, Perkins, Folds, & Golden, 1997), hypocortisolism may increase susceptibility of developing chronic inflammatory disease, autoimmune disease, and other diseases characterized by inflammation (Vanderhaeghe, 2001). Hypercortisolism has been associated with cognitive and memory impairment in older adults and individuals with depression, acute stress, and drug-induced hypercortisolemia (e.g., Keenan, Jacobson, Soleymani, Mayes, Stress, & Yaldoo,1996; Starkman, Gebarski, Berent, & Schteingart, 1992). It has also been associated with panic attacks (Bandelow, Wedekind, Pauls, Broocks, Hajak, & Rüthe, 2000), abdominal obesity (Bjorntrop & Rosmond, 2000), stuttering (Blood, Blood, Bennett, Simpson, & Susman, 1994), and schizotypal personality disorder (Weinstein, Diforio, Schiffman, Walker, & Bonsall, 1999). Hypercortisolism is a primary manifestation of Cushing's disease (Castro, Elias, Quidute, Halah, & Moreira,1999). Cortisol excess may also lead to insulin resistance, hypercholestrolemia, and hypertriglyceridemia, thereby contributing to the development of adult onset diabetes, cardiovascular disease, and hypertension (Gibson, Checkley, Papadopoulos, Poon, Daley, & Wardle, 1999).

Sensitization to glucocorticoid activity after repeated or extreme stress could result in upregulation of the number of glucocorticoid receptors, with increased negative feedback. This increased feedback decreases cortisol secretion and may lead to chronic hypocortisolism (Boyer 2000). Hypocortisolism has been found to be

associated with posttraumatic stress disorder (PTSD), healthy individuals living under ongoing stress, patients with stress-related bodily disorders (Heim et al., 2000a), girls with antisocial behaviors (Pajer, Gardner, Rubin, Perel, & Neal, 2001), rheumatoid arthritis (Neeck, Federlin, Graef, Rusch, & Schmidt, 1992), and chronic fatigue syndrome (Cleare et al., 2001; Demitrack et al., 1991). Passive coping styles, such as repression, avoidance, and denial, may also be related to hypocortisolism (Heim et al., 2000a).

Cortisol levels can be measured to assess the effects of interventions intended to reduce stress or HPA reactivity. For example, humor and laughter were found to lower cortisol levels (Berk 2000). Other studies used cortisol measurement as a means of assessing therapeutic response to corticosteroid treatment (Cleare, Heap, Malhi, & Wessely, 1999; DeBattista, Posener, Kalehzan, & Schatzberg, 2000). Another study that used cortisol measurement showed that short-term estradiol treatment could potentially lower cortisol levels in postmenopausal women toward more normal levels and may be able to restore feedback sensitivity of an aging HPA axis (Kudielka, Schmidt-Reinwald, Hellhammer, & Kirschbaum, 1999). Although further studies with larger samples are needed to substantiate findings of some of these studies, they suggest that cortisol measurement may be useful in assessing in a variety of stress-related health problems and outcomes.

Stress Hormone Measurement

Stress hormones can be measured as individual components of the stress response or collectively to gauge activation at different levels along the HPA axis. Many studies measure cortisol levels only, since cortisol is an easily accessible peripheral measure that has been shown to provide a reliable indication of HPA axis dysfunction (Baum & Grunberg, 1997) and offers other advantages that will be

discussed later in this article. However, measurement of the hormones secreted at each level of the HPA axis is useful in identifying the mechanisms that underlie HPA axis dysregulation (Nicolson, Storms, Ponds, & Sulon, 1997). Also, important in understanding HPA axis function is the differing information that can be acquired from either passive measurement of hormone levels (referred to as basal levels) or activating the system under controlled conditions (referred to as challenge studies). Analogous to the use of challenge studies to assess HPA axis function is the use of the treadmill in cardiology to assess cardiovascular function while the heart is forced to work hard; abnormalities may be revealed that may not be by using a stethoscope to listen to heart sounds at rest. Similarly, important abnormalities may be revealed under conditions that challenge the HPA system. Studies that examine changes in affinity and density of the various receptors involved in stress hormone systems (e.g., Yehuda, Lowy, Southwick, Shaffer, & Giller,1991) and those that examine changes in the diurnal pattern of cortisol release (e.g., Smyth et al., 1997) can also add important mechanistic information. To fully investigate potential underlying mechanisms involved in HPA axis activity or control, measures of both direct HPA axis hormones and those brain chemicals (AVP, serotonin, β-endorphin) that contribute to HPA axis activity would be required.

Challenge Studies

Challenge studies are designed to evoke specific biological responses under controlled conditions allowing for an assessment of stress system responsivity (Southwick, Yehuda, & Wang, 1998). They assess the functional integrity of the feedback system at various levels of the HPA axis (Nicolson et al., 1997). An important design issue in challenge studies in stress research is providing an appropriate stimulus that can activate an individual's response, such that measurement is possible.

It is unethical to place human beings in potentially dangerous or threatening situations in order to measure their neuroendocrine or psychological reactions. Thus, safe yet stressful stimulations must be created when naturalistic measurement during a stressful event would be impractical or impossible. Approaches include the presentation of evocative images, sounds, or movies and the administration of pharmacological agents to activate stress hormone systems.

The most well replicated challenge to the HPA axis is the dexamethasone suppression test (DST). Although first developed to identify Cushing's syndrome, it has been used extensively in depression studies since the early 1980's (Nelson & Davis, 1997). Historically, a 1 mg oral dose of dexamethasone, which acts as an agonist at glucocorticoid receptors, is given at 2300h. Plasma or salivary samples are taken the morning prior to the dose of dexamethasone and the subsequent morning and afternoon. However, more recently other doses of dexamethasone have been used (e.g., 0.25 mg, Reynolds, Bendall, Whorwood, Wood, Walker, & Phillips, 1998; 1.5 mg, Zobel, Yassouridis, Frieboes, & Holsboer, 1999). If HPA feedback regulation is functioning normally, cortisol secretion will be decreased or suppressed the next morning in response to the dexamethasone. If there is dysregulation of HPA feedback and cortisol levels are not decreased, the response is referred to as 'non-suppression' (Poland, Rubin, Lane, Hart, & Lesser, 1987). Non-suppression of cortisol after dexamethasone administration is seen in approximately one-half to two-thirds of patients with depression (Nelson & Davis, 1997). If there is an exaggerated suppression in cortisol levels in response to dexamethasone, it is referred to as 'supersuppression' or "hypersuppression". Exaggerated suppression of cortisol after dexamethasone administration have been observed in a number of traumatized populations, including combat veterans, (Yehuda, Southwick, Krystal, Bremner,

Charney, & Mason,1993) earthquake victims (Goenjian et al., 1996), and adults with a history of childhood sexual abuse (Heim et al., 2000b).

The combination of dexamethasone suppression followed by CRH stimulation is a more sensitive test for examining HPA axis function. For example, the combined dexamethasone/CRH test has been used to predict with a high degree of accuracy the risk of relapse in patients successfully treated for major depression (Zobel et al., 1999). Also, the dexamethasone-CRH combination challenge increased the sensitivity of the test in the diagnosis of Cushing's disease to greater than 85% (Yanovski, Yanovski, & Chrousos, 1993). The ACTH and CRH findings would not have been picked up if cortisol alone had been measured.

Cortisol, whether it is measured independently or in combination with other stress hormones and under basal or challenge conditions, is the most frequently measured neuroendocrine product in stress-related studies. Thus, it is important to have knowledge of the methodological approaches and interpretive issues in relation to cortisol measurement.

Methods and Issues in Cortisol Measurement

Cortisol can be measured in urine, plasma, and, more recently, saliva. Plasma, urinary, and salivary cortisol measures are highly intercorrelated (Weinstein et al., 1999). Salivary cortisol appears to be as sensitive a measure of stress reactivity as urinary and plasma cortisol (Weinstein et al., 1999). The fluid sampled will depend on the research question and design, the nature of the stressful event, and the logistics of collecting, preserving, and assaying samples.

Since cortisol is extensively metabolized to tetrahydrocortisone in the liver, a small amount of free cortisol and larger quantities of its metabolite are excreted in urine (Baum & Grunberg, 1997). Consequently, the metabolite is more readily available and

measurable in urine samples than is free cortisol. However, urinary excretion does not directly reflect adrenal activity since it is dependent on cortisol metabolism (Heim et al., 2000b) and urine excretion. The elimination half-life of cortisol is about 30 minutes (Baum & Grunberg). Urine collected immediately after an experimental session may reflect several hours of experience, since urinary cortisol and its metabolite are excreted slowly as the bladder fills (Baum & Grunberg). Twenty-four hour collection of urine, which is often difficult to complete, is needed to assess overall HPA axis activity. For these reasons, most studies measure either plasma or salivary cortisol.

In plasma, cortisol is present in unbound (free) and bound quantities. At least 90% of cortisol is bound to plasma proteins, with the largest portion being bound to corticosteroid-binding globulin and a lesser portion to albumin (Schimmer & Parker, 1996). Thus, normally less than 10% of cortisol is unbound and biologically active. Total cortisol levels, which reflect both bound and unbound cortisol, are frequently reported in the literature. Total plasma cortisol levels can be misleading if there are individual differences in level or activity of corticosteroid-binding globulin (Nicolson et al., 1997). For example, corticosteroid-binding globulin levels have been shown to be decreased in women with a major depressive episode (Heim et al., 2000b).

It has been established that it takes several minutes before an HPA response to a stressor becomes discernable in the periphery and that the effects of venipuncture-induced stress on plasma cortisol would not be evident for at least 7 minutes after the sample is drawn (Baum & Grunberg, 1997; Biondi & Picardi 1999; Kirschbaum & Hellhammer, 2000). Therefore, a baseline plasma cortisol sample can be drawn immediately after venipuncture (Baum & Grunberg). When venous samples are to be drawn repeatedly, an intravenous catheter with a stopcock can facilitate sampling and reduce venipuncture-related stress.

A major advantage of salivary sampling is that cortisol in saliva is 100% unbound and biologically active (Baum & Grunberg, 1997; King et al., 2000). Stress causes a reduction in salivary flow, which could alter the concentrations of some substances found in saliva. However, salivary flow has little or no effect on salivary cortisol levels, as the small size and high lipid solubility of cortisol molecules enable them to diffuse readily through cell membranes into saliva. Kirschbaum and Hellhammer (2000) showed that cortisol injected intravenously starts to appear in saliva in less than a minute. Salivary levels peak 1 to 2 minutes after plasma levels (Baum & Grunberg). Although saliva and plasma cortisol measures produce highly correlated results, cortisol concentrations are much lower in saliva than in plasma (Baum & Grunberg; Shimada, Takahashi, Ohkawa, Segawa, & Higurashi, 1995). However, ultra sensitive assays for cortisol are commercially available (Maniga & Golinsky, 2001). Since reference values for cortisol differ based on gender, age, pregnancy, use of hormonal therapies, and type of assay kit, the appropriate reference values must be selected for individual studies (Painter, Cope, & Smith, 2001).

Cortisol release shows clear circadian (pertaining to 24-hour clock) and diurnal (pertaining to daylight hours or daylight to dark) rhythms. Normally about 15 or more pulsatile bursts of cortisol are released in a 24-hour period in children and adults (Baum & Grunberg, 1997; Jett, Samuels, McDaniel, Benda, LaFranchi, & Reynolds, 1997; Knutsson et al., 1997). A characteristic episodic burst pattern is shown in Figure 3.2.

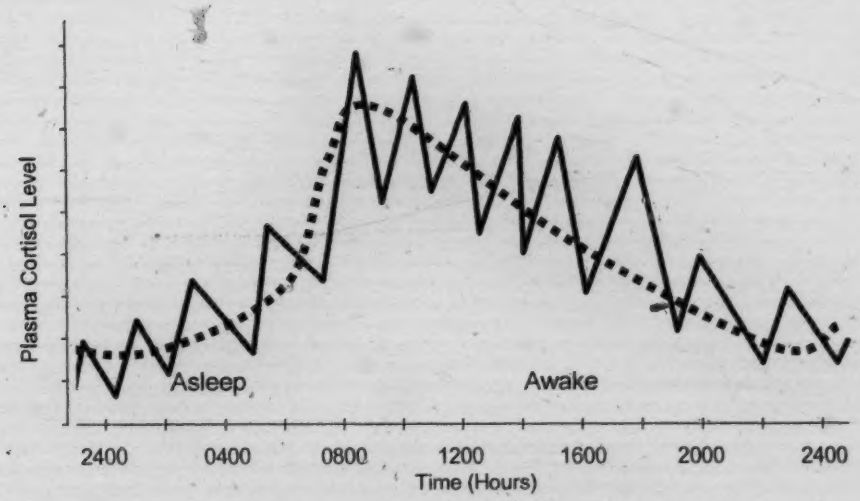

Figure 3.2. This is an example of an episodic "burst" pattern that is characteristic of the circadian release of cortisol. The largest burst during the 24 hour period occurs about 30 minutes after awakening for the day and the nadir occurs around midnight. Although it is not reflected in this diagram, the adrenal cortex is quiescent between bursts while cortisol stores are replenished. The duration of quiescent periods is not known.

Newborns and extremely low birth weight infants have been shown to lack a circadian pattern of cortisol release (Jett et al., 1997). Cortisol levels peak about one-half hour after awakening, with a 50% to 100% increase in cortisol levels, and reach their lowest point around midnight (Kirschbaum & Hellhammer, 2000). Both sleep-wake and dark-light transitions have been shown to be associate d with larger cortisol secretory pulses in healthy individuals (Caufriez, Moreno-Reyes, Leproult, Vertongen, Van Cauter, & Copinschi, 2002; Leproult, Colecchia, L'Hermite-Baleriaux, & Van Cauter, 2001; Scheer & Buijs, 1999). It is unclear whether circadian or diurnal rhythms of cortisol release are altered by certain health problems. For example, whereas Holsboer and Barden (1996) reported that diurnal rhythms were blunted in depression,

others (Osran, Reist, Chen, Lifrak, Chicz-DeMet, & Parker,1993; Young, Carlson, & Brown, 2001) found no evidence of altered diurnal rhythms in depressed women. Considered together, these findings suggest that timing of cortisol sampling is very important.

When multiple saliva samples are collected, times are often chosen to reflect differing points of the circadian or diurnal pattern of cortisol secretion. For example, one can be collected between 9 and 11 pm, when levels are normally low, and the other collected about one-half hour after waking up, when levels normally peak (King et al., 2000). Since saliva samples can be affected by food or smoking, saliva is collected prior to breakfast in the morning or after at least 30 minutes of not eating or smoking during the day (Maniga & Golinsky 2001). Although saliva can be expectorated directly into a container, a Salivette (Sarstedt Inc.) may be a more palatable collection device (Baum & Grunberg 1997) and used unless the assay protocol specifically indicates interference. A Salivette is a small plastic tube containing a cotton roll. When a Salivette is used, an individual is typically instructed to remove the cotton roll, chew it for a full minute, and then replace it in the Salivette. Once the specimen is obtained, the Salivette is centrifuged. The saliva can then be aliquoted and stored at –80C until analyzed. A variety of temperature conditions and time lags between obtaining the saliva sample and centrifugation show no significant differences in cortisol values, thus demonstrating the stability of cortisol (Baum & Grunberg).

Advantages of Salivary Measurement

Saliva sampling offers a number of advantages over other collection methods. It is noninvasive, painless, less stressful, and more easily performed (Hofman 2001). It is also less expensive, raises fewer ethical concerns than more invasive methods, and has higher rates of compliance (Baum & Grunberg, 1997). As indicated earlier, the

level of salivary cortisol directly reflects the amount of cortisol available to interact with its receptors. This allows for easier interpretation of results as compared to plasma sampling, where the amount of corticosteroid binding globulin and influences of concomitant drug action must be considered. Venipuncture, which may result in stress-related increases in overall levels, can be avoided (Reynolds et al., 1998). In addition, multiple sampling over the day or many days can be completed at home, thus increasing the feasibility of doing longitudinal studies. Saliva samples have been obtained without difficulty from children (Shimada et al., 1995) and people with poor cognitive skills, such as those with Alzheimer's (Samuels, Furlan, Boyce, & Katz, 1997). Salivary sampling may also be a useful alternative in situations in which venous sampling may be undesirable, such as in individuals with integumentary disorders or burns. However, saliva sampling may be more difficult in individuals who have limited chewing ability and pre-term infants. It may be that a small sterile syringe or similar device could be used to collect saliva in such situations, if deemed safe. Kirschbaum and Hellhammer (2000) suggest that a few crystals of sugar-sweetened Kool-Aid ™ may help to increase saliva volume in infants and small children. It may also be useful in older individuals in whom salivary flow is inadequate, although testing to ensure that this does not influence cortisol results is recommended. The use of citric acid or other products that could lower saliva pH are not recommended, since low pH samples may lead to falsely elevated cortisol values upon assaying (Kirschbaum & Hellhammer).

In summary, many important cortisol changes associated with stress can be measured relatively easily in blood, urine, and in particular saliva. However, irrespective of which tissue is sampled, cortisol levels may be influenced by any of several factors.

Factors Influencing Cortisol Levels

A review of the literature indicates that a number of factors may influence cortisol levels. Many of these must be considered in the design, selection of variables, and interpretation of results of studies measuring cortisol. Important factors include age, gender, menstrual phase, estradiol levels, drugs, physical activity, sleep, diet, obesity, and anticipatory stress.

The findings in regard to age are not consistent. It has been suggested that although older adults have approximately the same size bursts of cortisol secretion as younger adults, it may take longer for their cortisol levels to return to baseline after a stressor and they may secrete more stress related hormones in their normal non-stressed states (Sapolsky 2000b). Age-related changes in specific cytochrome isoenzymes involved in steroid metabolism may affect baseline cortisol levels. However, a number of studies did not support this hypothesis. A study examining the effect of age on levels of cortisol in response to a psychosocial stressor found that while basal cortisol levels were moderately increased, cortisol responses to the stressor did not increase in either magnitude or duration in the elderly (Nicolson et al., 1997). Other studies found a lack of age-related changes in basal cortisol levels (e.g., King et al., 2000; Kudielka et al., 1999). However, a study using a dexamethasone-CRH combined challenge found that the elderly had higher ACTH peaks and more pronounced cortisol responses (Kudielka et al., 1999). Another study showed interperson variability but intraperson stability of baseline plasma cortisol concentrations in response to a low dose of dexamethasone in elderly individuals (Huizenga, Koper, de Lange, Pols, Stolk, & Grobbee, 1998). Age may be a particularly important factor to consider when measuring cortisol in infants. Small for gestational

age but healthy infants have been shown to have significantly higher cortisol values than appropriate size for gestational age healthy infants (Heckmann et al., 1999).

Investigations regarding potential gender differences have also produced equivocal results. Roelfsema et al., (1993) found that men and women have equivalent daily cortisol secretion rates, despite the finding that men secrete more ACTH than women to maintain this equivalency. King et al., (2000) found no gender differences in morning or evening basal salivary cortisol levels in 147 health volunteers sampled four times in one year. However, estradiol use and menstrual phase were not controlled. Significant differences in cortisol levels in relation to menstrual phase and estradiol levels in females have been reported, particularly when conducting challenge tests. Decreased basal cortisol concentrations and enhanced suppression of cortisol by dexamethasone have been found in women in the follicular phase of the menstrual cycle (Altemus, Redwine, Leong, Frye, Porges, & Carter, 1997; Heim et al., 2000a). Kirschbaum et al., (1999) determined that gender, menstrual cycle phase, and oral contraceptive use bore important effects on HPA responsiveness to psychosocial stress in healthy subjects, with salivary cortisol being greatly reduced in oral contraceptive users after HPA axis challenge. It is important to note that none of these differences emerged in total plasma cortisol levels and, therefore, salivary cortisol levels may be more sensitive measures of cortisol reactivity in studies of HPA function in women.

A number of drugs, such as some herbal products, substances of abuse, corticosteroids, sex hormones, and antidepressants, may influence cortisol levels. Some specific drugs that may increase cortisol levels include amphetamines, estrogens, ethyl alcohol, lithium, methadone, nicotine, and spironalactone (Wilson, 1999). Drugs that may decrease cortisol levels include androgens, barbiturates,

dexamethasone, levodopa, and phenytoin (Wilson). Consequently, studies that measure cortisol frequently exclude individuals who have taken drugs in preceding weeks that could alter mood or cortisol levels (e.g., Mokran, Duval, Crocq, Bailey, & Macher, 1997).

Some studies have focused on the effects of physical activity on cortisol levels. Salivary cortisol levels increased when healthy volunteers were standing, but not when they were sitting or lying, during sample collection in one study (Hennig, Friebe, Ryl, Kra¨mer, Bo¨ttcher, & Netter, 2000). Yet plasma cortisol levels did not change appreciably as a result of exercise in convenience samples of pre-, peri-, and post-menopausal women (Clearlock & Nuzzo, 2001). In another study that compared exercisers who used oral contraceptives to those who did not, those who used oral contraceptives had lower unbound plasma cortisol levels (Kirschbaum, Platte, Pirke, & Hellhammer, 1996). Further investigation of the effects of physical activity and hormonal therapies on cortisol levels is needed.

Scheduling the time of cortisol collection is important, since time of year and time of day may influence results and thus decrease comparability across studies. Seasonal differences in which winter plasma cortisol levels are significantly higher than summer levels have been reported (King et al., 2000). Diurnal rhythm in cortisol levels is well established. Salivary cortisol levels characteristically peak about 30 minutes after morning awakening with low points late afternoon and late evening (King et al.). Different researchers have used different protocols for morning cortisol sampling (i.e., on awakening, at 0800h, 30 minutes after awakening), thus the data among studies are not directly comparable without considering the specific times of data collection. In planning research that will include salivary sampling of cortisol, it is important to determine a specific time of sample collection. Important factors that should be

considered when determining the time for collection are comparability to published data and study objectives (i.e., whether the focus is on peak values, changes in response to a challenge test, etc.)

Cortisol responses to awakening under particular conditions have been the focus of some studies. For example, postmenopausal women showed the characteristic salivary cortisol increase after awakening, whether or not they took estradiol-containing medication (Kudielka et al., 1999). Significant increases in salivary cortisol were found in response to both nocturnal and morning awakening (Hucklebridge, Clow, Rahman, & Evans, 2000) and after one night of sleep deprivation, especially at 1:30 p.m. the next day (Goh, Tong, Lim, Low, & Lee, 2001). Caufriez et al. (2002) found in a small group of subjects that sleep onset was consistently followed by a decrease in plasma cortisol concentrations while both sleep-wake and dark-light transitions were consistently associated with heightened cortisol secretion.

Feeding and digestion patterns may also influence cortisol levels and have implications for scheduling collection times. Salivary cortisol has been reported to increase more after the midday meal than after other meals and after a high protein meal (Gibson, Checkley, Papadopoulos, Poon, Daley, & Wardle, 1999). Gastrointestinal transit time may be prolonged significantly in the second half of the menstrual cycle, possibly an effect of the gonadal hormones, and this may alter drug absorption rates and bioavailability (Gregory, 1999). The latter finding is further evidence of the importance of controlling for menstrual phase when cortisol is measured in response to pharmacological therapy or a challenge. Another related issue is abdominal obesity, which has been associated with sensitization of the HPA axis (Heim et al., 2000a) and greater cortisol secretion (Epel et al., 2000).

Pain experience may influence cortisol levels. Acute pain may act as a stressor and increase cortisol levels. However, people with chronic pain and teachers with a high number of physical complaints have shown exaggerated cortisol suppression in response to dexamethasone and reduced salivary cortisol levels (Heim et al., 2000a).

Although there are only a few reports of the effects of environmental and contextual factors, such as room temperature, odors, darkness, safety concerns, background noise and familiarity with the setting on cortisol levels per se, these factors are likely to be important in the design of any study that measures stress or anxiety. Use of a naturalistic rather than an institutional setting may be feasible with salivary cortisol collection and could help to reduce effects of environment-induced stress. For example, in one study, sufferers of panic attacks collected saliva samples during naturally occurring attacks wherever and whenever they occurred, rather than measuring their responses to chemically induced panic attacks in a clinical setting at a prearranged time (Bandelow et al., 2000).

When the cortisol measurement design includes multiple sampling, the potential physiological and psychological responses of subjects to repeated collection should be considered. King et al., (2000) found a significant decrement in HPA axis response and stabilization in cortisol levels over time that they attributed to habituation to repeated saliva collection with subsequent blunting of anticipatory stress. They further suggested that anticipatory stress is likely to be greater when plasma rather than saliva is sampled. Others have reported similar findings (e.g., Weinstein et al., 1999). At least initially, anticipation of the novelty, discomfort or inconvenience of collection may activate the HPA axis and increase cortisol levels (Weinstein et al.).

Summary

In summary, changes have been observed in stress hormone systems in relation to a number of psychological and physical disorders. Cortisol as a peripheral reflection of HPA axis activity possesses several properties that make its measurement highly useful for investigating the biological effects of stress. Although cortisol can be measured relatively easily in blood, urine, and saliva, saliva sampling has a number of advantages. A number of factors that could potentially influence cortisol levels have been identified. Unfortunately, not all published studies that included cortisol measurement controlled for such factors, leading to interpretive concerns and limitations in the generalizabilty of the data.

There is still much that can be learned from incorporating neuroendocrine measurement into multidimensional studies examining the impact of stress on health and the impact of nursing interventions aimed at supporting successful management of stressors. With well-considered attention to controlling the factors that can affect collection and analysis as well as controlling for individual factors that affect HPA axis activity, data from neuroendocrine studies can contribute to our understanding of the complex relationship between health and stress. As nursing increasingly searches for bodies of knowledge that can provide the basis for evidence-based practice, data regarding the biological effects of chronic or extreme stress can add significantly to a holistic approach to stress-related research.

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CHAPTER FOUR

The Influence of Menstrual Phase on Startle Reactivity in Healthy Women

The startle eyeblink response paradigm is used increasingly as a robust and reliable tool for evaluating the startle reflex component of the stress response system. Despite the proliferation of studies measuring startle in health and illness conditions in both genders, differences in startle responses across phases of the menstrual cycle in women have yet to be determined. This information is critical for the interpretation of research studies that assess startle responses. In order to address this need, the present study compared startle responses in healthy women during the follicular and luteal phases of their menstrual cycles.

The startle reflex is a protective response that develops in early infancy in healthy humans. In humans, the acoustic startle eyeblink component of the startle reflex is commonly used to investigate the neurobiological basis of stress-related disorders. The acoustic startle response is mediated by a pontine-based neural circuit and involves the protective, reflexive contraction of skeletal muscles in response to an abrupt acoustic stimulus (Yeomans, Li, Scott, & Frankland, 2002).

The startle response is measured as an index of central and peripheral and autonomic reactivity (Morgan, 1997). Numerous studies have measured startle in men and women. However, studies that have assessed or controlled for potential menstrual phase differences in acoustic startle reactivity in women are few. Several studies have assessed the effects of menstrual cycle phase on heart rate, which is also measured as an index of autonomic activity. Most researchers failed to find menstrual cycle phase effects on heart rate responses to acute stressors in healthy women (Dean,

Perreault, Mazzeo, & Horton, 2003; Fontana & McLaughlin, 1998; Girdler, Pedersen, Stern, & Light, 1993; Hirshpren, Tzoran, Makrienko, 2002; Litschauer, Zauchner, Huemer, Kafka-Lutzow, 1998; Mills et al., 1996; Spielmann, 2004; Stoney, Owens, Matthews, Davis, & Caggiula, 1990). In contrast, three studies reported higher heart rate in luteal as compared to follicular phase women (Manhem, Jern, Pilhall, Shanks, & Jern, 1991;Tanaka, Sato, Umehara, & Nishikawa, 2003; Yamamotoa, Tsutsumib, Furukawaa, Kannoa, Maruyamaa, & Satohb, 2003). McFetridge and Sherwood (2000) reported lower plasma catecholamine levels, systemic vascular resistance, and heart rate in luteal phase women. Differences in sample size, precise timing of measurements, equipment used to measure physiological parameters and inter-subject variability across the studies may account for the equivocal results. Clearly, the potential influence of menstrual phase on autonomic parameters requires further investigation.

Amplitude, habituation, and prepulse inhibition (PPI) of the startle eyeblink response are stable measures within individuals (Cadenhead, Carasso, Swerdlow, Geyer, & Braff , 1999). Amplitude refers to the size of the physiological startle response to an abrupt loud stimulus. Abnormalities of startle amplitude have been associated with stress-related disorders, such as panic disorder (Larsen, Ruffalo, Nietert, & Davidson, 2001) and posttraumatic stress disorder (PTSD) (Medina, Mejia, Schell, Dawson, & Margolin, 2001). Exaggerated startle is a DSM-IV diagnostic criterion for PTSD (American Psychiatric Association, 1994). Whereas habituation, as indicated by decreasing startle amplitude to repeated presentations of the same stimulus, occurs in healthy individuals, this process may be impaired in PTSD (Shalev, Peri, Brandes, Freedman, Orr, & Pitman, 2000). PPI occurs when a weak acoustic stimulus preceding a startle eliciting stimulus results in diminished startle amplitude. PPI is hypothesized to

protect healthy humans from sensory overload by enabling them to attend to salient stimuli and 'gate' or filter out extraneous information (Graham, 1975; Thorne, Dawson, & Schell, 2005). PPI has been found to be significantly impaired in disorders such as schizophrenia (Braff & Geyer, 1990) and PTSD (Filion, Dawson, & Schell, 1998; Graver, 2000; Metzger, Orr, Berry, & Ahern, 1999; Shalev et al.).

Several studies have assessed the influence of gender in modulation of the startle response. One of the most frequent findings regarding gender differences in the acoustic startle response in healthy humans is that women exhibit reduced sensorimotor gating, as evidenced by reduced PPI, compared to men (Aasen, Kolli, & Kumari, 2005; Kofler, Muller, Reggiani, & Valls-Sole, 2001; Kumari, Gray, Gupta, Luscher, & Sharma, 2003). Conversely, Ludewig, Ludewig, Seitz, Obrist, Geyer, and Vollenweider (2003) found no effects of gender on PPI. Few of these studies assessed or controlled for menstrual phase effects on startle. Findings of gender differences in startle modulation may be reflecting menstrual phase fluctuations in female sex hormones and other neurochemicals.

Some normal variability in PPI may occur in healthy women due to hormonal fluctuations across the menstrual cycle (Jovanovic et al., 2004). Only two studies were found that assessed menstrual phase effects on startle reactivity in humans. One was a cross-sectional study that found luteal phase women have reduced PPI as compared to follicular phase women (Swerdlow, Hartman & Auerbach, 1997). The only study found that assessed within-subject (n = 14) menstrual phase effects on startle reactivity also reported that PPI was reduced in luteal women (Jovanovic et al.). These authors found no menstrual phase effects on startle magnitude or latency.

The central neural mechanism hypothesized for menstrual phase effects on PPI is that estrogen stimulates the synthesis and release of dopamine in the striatum

(Becker, 1999). Dopamine levels may subsequently be increased at times of the menstrual cycle when estrogen levels are higher. Because increased striatal dopamine levels are associated with reduced sensorimotor gating or PPI (Swerdlow, Wasserman, Talledo, Casas, Bruins, & Stephany, 2003), PPI may be decreased in the ovulatory (days 13 to 15) or early luteal phases. Gogos, Nathan, Guille, Croft, and van den Buuse (2006) found that exogenous estrogen did not affect startle amplitude, habituation or PPI at 30 or 60 ms interstimulus intervals (ISI) in 11 healthy women; however, they found that estrogen significantly prevented PPI at 120 ms ISI from being diminished by activation of specific serotonin receptors . These findings suggest that the relationships among estrogen, dopamine and other neurotransmitters involved in startle circuitry are complex and require further study.

The purpose of the present study is to determine whether menstrual phase effects are sufficient to be reflected in changes in startle amplitude and prepulse inhibition, by comparing startle responses in healthy women during the follicular and luteal phases of their menstrual cycles. This information will contribute to the general understanding of the neurobiology of arousal. Also, it will help to identify gender-specific factors that may be important to consider in interpretation of research findings based on startle measurement.

Method

Participants

Twenty-two women [mean age = 29.4 years] signed informed consent forms approved by the institutional ethics review board. Two of the 22 women became ineligible prior to the second assessment (commenced medication therapy – one oral contraceptive, one corticosteroid) and were excluded from phase comparisons. Twenty

women [13 follicular and 7 luteal at their initial test session] were retested during the alternate phase of their menstrual cycle at the same time of day as in their first test. The range of days since onset of last menstrual period was 5 to 10 for the follicular phase and 22 to 27 for the luteal phase. All participants spoke English and had regular menstrual cycles. All except one had post-secondary education. None of the participants were pregnant, breast-feeding or using oral contraceptives or other hormonal preparations. None were taking any other drugs, including herbal products, street drugs, or alcohol, on a regular basis or within the 24 hour period preceding testing. Only one participant consumed more than 3 cups of caffeinated beverage on a daily basis and smoked cigarettes. None ingested caffeinated beverages or smoked cigarettes within 2 hours of testing. None had a history of depression, anxiety disorders, or other DSM-IV Axis I psychiatric disorders [as determined by the *Mini International Neuropsychiatric Interview (MINI;* Sheehan et al. 1997)], active medical or neurological condition, or self-reported hearing deficit [inability to hear sounds in range of 45 to 114 decibels]. Immediately prior to startle testing, participants marked a point on visual analogue scale to indicate current perceived stress level (1 = least stress to 10 = most stress ever experienced).

Startle Protocol

A commercial computerized acoustic startle response (ASR) system (San Diego Instruments Inc., San Diego, CA, USA) with an infrared eyeblink registration module was used to measure the startle response. The system delivers binaural acoustic stimuli via headphones. The ASR signal was filtered at 1000 Hz. Headphones were placed in alignment with the auditory canal and amplifier gain settings were held constant to promote consistency in acoustic signal intensity across participants and test sessions. To enable noninvasive, simultaneous bilateral measurement of the

eyeblink response, subjects wore safety eyeglasses with lenses that are fitted bilaterally with infrared transceivers. An infrared beam directed onto the cornea is reflected back to the sensor when the eye blinks. Thus, the system measures reflectance rather than electromyography. Participants sat in a recliner chair with their eyes open and looking straight ahead. The assessment room temperature and lighting were constant. Acoustic stimuli were bursts of white noise (40 ms duration, 100 dB intensity) with instantaneous onset over continuous background white noise (70dB) through headphones binaurally. Although researchers have used a range of startle-eliciting acoustic stimuli (e.g., 110 dB: Shlik, Zhou, Koszycki, Vaccarino, & Bradwejn, 1999; 115 dB: Light & Braff, 2001), Shalev et al. (2000) were able to induce startle satisfactorily at 95 dB. Background noise typically ranges from 60 dB (e.g., Light & Braff) to 75 dB (e.g., Shalev et al.).

Since this study included pulse alone and prepulse trials, the protocol for stimulus delivery established by Cadenhead, Swerdlow, Shafer, Diaz, & Braff (2000) was used. The protocol was replicated as described by Cadenhead et al. (2000), except that the maximum stimulus intensity used was 100 dB rather than 115 dB. Also, the prepulse trials were in accordance with the recent modification of the prepulse component of the protocol (Cadenhead & Schafer, 2002, personal communication). Each startle session began with a five minute acclimation period for participants prior to testing. A 70-dB background noise was delivered and continued constantly throughout the acclimation period and test session. The startle protocol used three 'trial' types: pulse alone, prepulse followed by pulse, and no stimulus. A pulse (a 100 dB burst of white noise for duration of 40 ms) was presented either alone or following a prepulse (an 86 dB burst of white noise for duration of 20 ms). When used, a prepulse preceded

a pulse by 30 ms, 60 ms, or 120 ms interstimulus intervals [ISI] in pseudorandom order. Intertrial intervals ranged from 8-22 seconds in pseudorandom order.

A startle test session was comprised of four consecutive blocks of trials. The first and last blocks each consisted of 5 pulse alone trials. The second and third blocks each included prepulse trial types (6 trials each of 30, 60, 120 ms ISI) that were sequenced and intermixed with pulse alone (12 trials) and no stimulus (12 trials) trial types in a set pseudorandom pattern. A complete startle session was 58 trials (116 eyeblink responses assessed per subject). The total time for a startle session was 25 minutes.

Although evidence of a robust circadian pattern of acoustic startle was reported in rats (Chabot & Taylor, 1992; Frankland & Ralph, 1995), it is not known whether there may be a circadian rhythm of startle in humans. Exogenous corticosteroid was found to influence startle in a study of healthy volunteers (Buchanan, Brechtel, Sollersili, & Lovallo, 2001); however, it is not known whether circadian fluctuations in endogenous cortisol levels are sufficient to influence startle. Subsequently, the present study assessed startle at the same time of day for both phases for each subject to control for potential circadian effects.

Digital ASR-eyeblink signals were full-wave rectified and smoothed by an averaging routine set to calculate a rolling average (smooth) of 10 data points. The system was set to record at a rate of 1000 samples per second starting 200 milliseconds before each pulse or prepulse stimulus. The 200 ms pre-recording permitted identification, during trial-by-trial visual inspection of right- then left-eyeblinks, of any extraneous voluntary or spontaneous eyeblinks (defined as baseline shift greater than 35% of maximum in the absence of a stimulus or when onset and peak latencies differ by > 95 ms in stimulated responses) that may contaminate ensuing

stimulus responses. Fewer than 5% of trials were subsequently excluded on this basis. Startle data scoring was both computer-assisted (EMG Data Reduction Software, San Diego Instruments Inc., San Diego, CA, USA ,2003) and manual in accordance with response quantification methodology described by Blumenthal, Cuthbert, Filion, Hackley, Lipp, and Van Boxtel (2005, p. 10). Consistent with these recommendations, all trials for all subjects were scored by one rater to avoid inter-rater variability. To increase reliability, every trial was scored twice, independently of previous scoring, by the same rater. Trials with the baseline shift greater than 20 percent of maximum were rejected by the software (recommended default setting, San Diego Instruments Inc.). In cases where an eyeblink waveform showed multiple peaks, the maximum value was identified as the peak.

Study Design & Data Analyses

A repeated-measures/crossover design was used. Participants were assessed twice, once during each menstrual phase. Microsoft Excel, SPSS 10.0.7, and EMG Data Reduction Software were used for data analysis. Raw data were coded and entered in accordance with established protocols for software and standardized instruments. Descriptive statistics were used to describe the demographic characteristics of the sample, extraneous variables and perceived stress. Left and right startle eyeblink data were averaged for each trial type within each block. In accordance with the startle protocol, three participants who exhibited a relative lack of startle reactivity, resulting in an excessive number of rejected trials, at one startle session each were considered "non-responders" and excluded from startle phase analyses. This is a lower non-responder rate than the 25% to 31% rate that may be considered typical (Cadenhead et al., 2000).

The dependent variables analyzed were: perceived stress, startle amplitude, habituation, peak latency, latency facilitation, and PPI. A visual analogue scale created for this study was used to assess perceived stress level. Immediately prior to each startle test, participants marked a point on a 10 cm line scale from 0 (least stress) to 10 (most stress) to indicate current perceived stress level. Startle amplitude (mV) was determined by averaging startle responses. Habituation was quantified by calculating the arithmetic difference of amplitude in mV for pulse alone trials in blocks 1 and 4 (pulse alone block 1 – pulse alone block 4). The time required to generate the peak of the first amplitude waveform, in which the baseline value shifted at least 10% above baseline, was used to determine peak latency (ms) for each pulse alone trial. Peak latencies were then averaged for each block of pulse alone trials. The mean of pulse alone trials minus the mean of prepulse trials was used to quantify latency facilitation (defined as the reduction in latency in ms by a prepulse). PPI was computed using difference scores: arithmetic difference between mean of pulse alone trials and mean of each of the prepulse conditions [(pulse alone) – (prepulse + pulse)]. For comparative purposes, PPI was also computed using percentage scores: arithmetic difference between mean of pulse alone trials and mean of each of the prepulse conditions divided by the mean of pulse alone trials multiplied by 100 [[(pulse alone) – (prepulse + pulse)]/(pulse alone)*100]. Although percentage scores correct for individual variability, difference scores have been considered the best way to report PPI (Cadenhead et al., 2000).

Correlational statistics and paired t-tests were used for within-subject and between-groups comparisons by block, trial type and menstrual phase. Multiple regressions with one-way ANOVA were used to identify factor contributions. An α of 0.05 was used for all analyses.

Results

Sample sizes vary among statistical analyses, since data were missing for some assessment components for a few subjects. Two of the 22 women who completed the first menstrual phase assessment became ineligible prior to the second assessment and were subsequently excluded from phase comparisons. In addition, blocks of startle trials with no stimulated eyeblinks were excluded from analyses of those blocks.

Perceived stress

Within-subject self-rated stress levels in the follicular and luteal phases were highly correlated (paired samples t-test: n = 20, $r = 0.723$, $p = 0.000$), indicating that individual's perceived stress levels did not vary by menstrual phase. However, between-subject self-rated stress levels ranged widely (luteal 0.2 to 6.9; follicular 0.4 to 5.0). Despite this inter-individual variability, the group means were similar (luteal mean 2.8, *SD* 1.92; follicular mean 3.0, *SD* 1.47). Some women were tested first in the follicular phase, and some were tested first in the luteal phase. When the effects of testing order were controlled, being tested first in the follicular phase was related to higher levels of perceived stress ($df = 19$, $F = 7.161$, $p = 0.015$; $r = -0.523$). Being tested first in the luteal phase had no effect on perceived stress.

Startle amplitude

Stimulated eyeblink amplitude was significantly greater than non-stimulated (spontaneous or voluntary) eyeblink amplitude during the first two trial blocks for both menstrual phases (follicular: $t = 4.117$, $df = 16$, $p = 0.001$; luteal: $t = 3.832$, $df = 16$, $p = 0.001$), indicating that acoustic startle probes were effective. Trials with non-stimulated eyeblinks or no response were excluded from subsequent data analyses.

Follicular and luteal phase women did not differ in startle amplitude in response to pulse alone stimuli, either within or between groups in any of the four blocks of trials. Controlling for menstrual phase at the first session, caffeine intake, and other extraneous variables did not yield significant differences.

Habituation

Normal habituation was observed across the four blocks (see Figure 4.1) in both follicular and luteal phase women. Paired differences were significant (follicular: $t = 4.418$, $df = 15$, $p = 0.000$; luteal: $t = 3.174$, $df = 15$, $p = 0.006$), indicating significant habituation across the test session occurred in both menstrual phases. Mean habituation of startle amplitude across the test session was highly correlated in follicular and luteal phase women ($n = 16$, $r = 0.614$, $p = 0.011$), indicating that there are no menstrual phase differences in habituation.

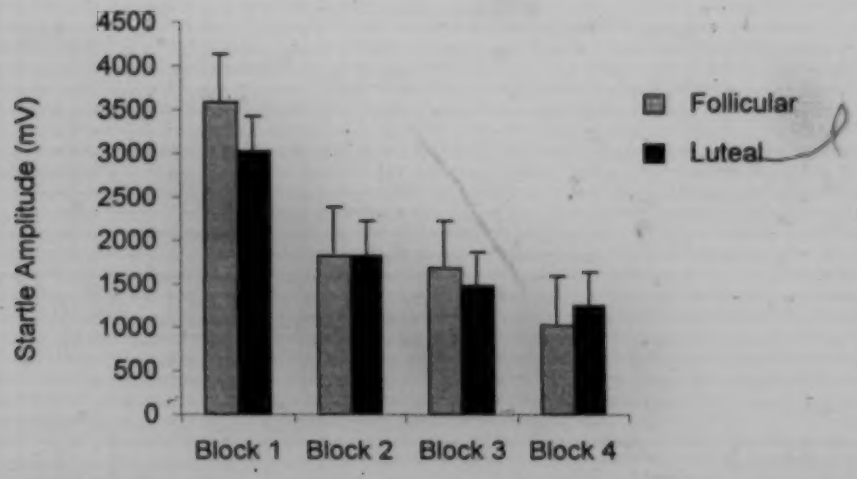

Figure 4.1. Relationship between menstrual phase and startle amplitude and between menstrual phase and habituation.

Latency and latency facilitation

A stable pattern of peak latencies for each trial type were observed within subjects. Peak latencies for each trial type within each block were averaged and compared statistically. Latencies for pulse alone trials by block are presented in Figure 4.2. No menstrual phase differences within or between subjects were found for either latency or latency facilitation.

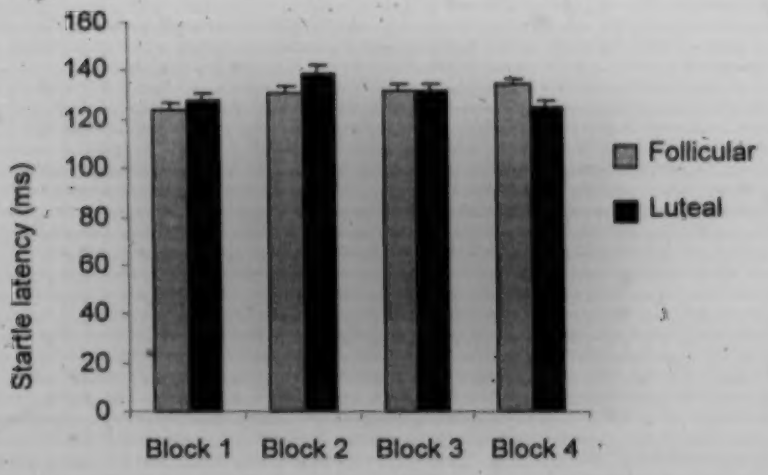

Figure 4.2. Relationship between menstrual phase and startle latency.

Prepulse Inhbition (PPI)

Prepulse inhibition of startle amplitude (sensorimotor gating) was assessed for three prepulse conditions: 30, 60, and 120 ms ISI. Luteal and follicular phase women demonstrated decreased startle amplitude in response to each of the 30, 60, and 120 ms ISI conditions. In both menstrual phases and both trial blocks, inhibition was greatest with the 30 ms ISI prepulse (see Figure 4.3). However, PPI was significantly greater at 30 ms ISI than 60 ms ISI only within follicular women (block 2: $t = -2.25$, $df =$

15, $p = 0.040$; block 3: $t = -2.49$, $df = 15$, $p = 0.026$. Menstrual phase differences in PPI were not found, using either difference scores or percentage reduction scores. Paired samples correlations were significant (range: $r = .527$ to $r = .815$, $p = 0.000$ to 0.036) across all ISI and trial blocks. These findings indicate that there are no menstrual phase effects on sensorimotor gating.

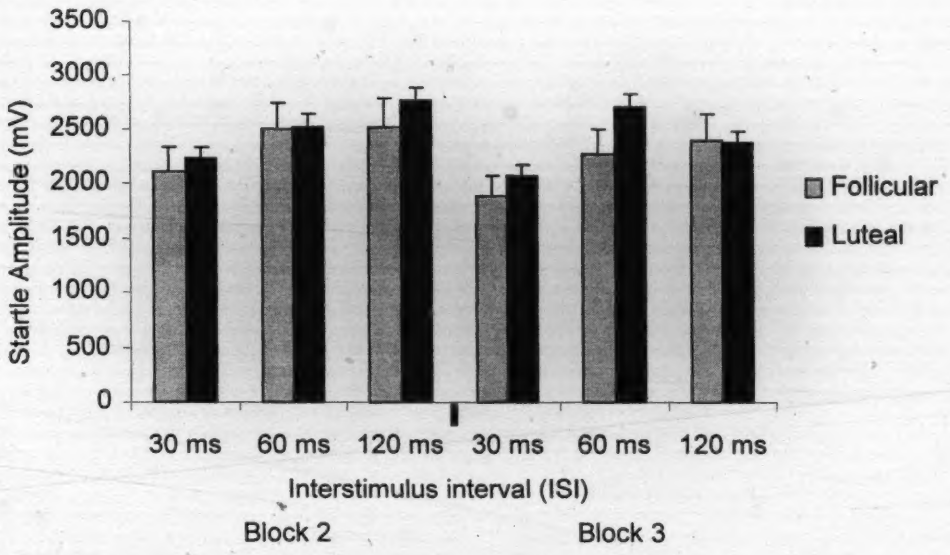

Figure 4.3. Relationship between menstrual phase and startle amplitude in response to each of the 30, 60, 100 ms ISI prepulse conditions.

Discussion

Cognitive process-oriented stress models imply that, when asked, individuals should be able to make reasonably accurate appraisals or judgments about how stressed they are (Cohen, Kessler, & Gordon, 1997; Folkman, Lazarus, Dunkel-Schetter, DeLongis, & Gruen, 1986; Graydon, 1984). In this study, the lack of a correlation between perceived stress level and startle amplitude suggests that perceived stress levels may not accurately reflect physiological stress parameters. The

within-subject stability and between-subject variability in perceived stress across menstrual phases may reflect individual differences in anxiety related to the use of unfamiliar equipment for startle assessment or other unknown factors. When the effects of testing order were controlled, the group tested first in the follicular phase showed significantly higher levels of perceived stress. This finding was unexpected in that luteal phase increases in tension, irritability and sensitivity to environmental stressors, all of which might increase general levels of perceived stress, are well documented (Ramcharan, Love, Fick, & Goldfien, 1992). Perhaps subjects limited their estimates of perceived stress to the acoustic startle test itself. Although testing order was not an influencing factor on other variables, the finding that perception of stress did vary by phase suggests that it may be important to assess premenopausal women during the same menstrual phase in stress-related studies.

The present study provides evidence that amplitude, habituation, latency and PPI of the acoustic startle response are stable within-subject neurobiological measures in a healthy premenopausal female population. The findings that menstrual phase exerted no effect on startle amplitude, habituation, latency or latency facilitation are consistent with those of Jovanovic et al. (2004). Inhibition was greatest with the 30 ms prepulse in both follicular and luteal women. The finding that PPI in follicular phase was significantly correlated with PPI in luteal phase contrasts with the finding of Jovanovic et al. that PPI was significantly reduced in the luteal phase in 14 healthy women. The reason for this difference in findings is not clear. Both studies assessed within-subject correlations. Whereas Jovanovic et al. used percentage scores to determine PPI, the present study used both percentage and difference scores to enable comparison with other studies that used either procedure. Also, the present study used a slightly larger sample. Differences in prepulse parameters may have influenced results. Jovanovic et

al. used a 20 ms burst of 85 dB pure tones at 50, 80, and 140 ms ISI. The present study used a 20 ms burst of 86 dB white noise 30, 60, and 120 ms ISI. These differences appear small, but guidelines for startle probes suggest that small differences can influence results and that white noise is a "more effective startle stimulus than is pure tone" (Blumenthal et al., 2005, p. 3). Sample sizes and differences in eyeblink recording devices may also affect results. Whereas the more common surface electrodes that record unilateral orbicularis oculi muscle activity were used by Jovanovic et al., the present study used safety glasses with sensors that simultaneously recorded bilateral eyeblink data. Surface electrode sensors may be more sensitive in detecting weak eyeblinks than methods dependent on overcoming the inertia of eyelid muscles (Flaten, 1993; Blumenthal et al., 2005). The corneal reflective sensors as used in the present study depend on movement of the larger heavier levator palpebrae muscle in the upper eyelid as well as the orbicularis oculi.

A potential limitation of this study is that some participants may have experienced anxiety related to test equipment or unknown sources that was sufficient to impact startle reactivity. However, the perceived stress analogue scale that was used to control for this factor suggests that perceived stress levels within individuals were stable across menstrual phases.

Strengths of the present study are the inclusion of a pre-stimulus recording period and the manual scoring method that was used to assess and score startle data. This rigorous process enabled identification and exclusion of non-stimulated eyeblinks that might otherwise have contaminated startle data. Another strength of this study is that potential circadian rhythm effects on startle were controlled.

In conclusion, findings of the present study suggest that menstrual phase does not affect startle amplitude, habituation and prepulse inhibition in healthy women.

Further within-subject investigations in larger samples of healthy women are needed to verify these findings. If verified, the design of clinical studies could be simplified. However, initial investigations would be needed to clarify whether menstrual phase fluctuations in female hormones can influence the startle reflex in clinical populations, such as those with stress related disorders. Comparisons of startle responses in healthy women at more points in the menstrual cycle, such as when estrogen levels and progesterone levels are highest and lowest, would be useful to assess potential contributions of specific hormones. These data and further planned studies contribute to the general understanding of the neuroendocrine basis of stress disorders and interpretation of research findings based on startle measurement in pre-menopausal women.

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CHAPTER FIVE

A Prospective Study of Physiological Hyperarousal and Coping as Correlates of Symptoms of Acute Stress Disorder and Posttraumatic Stress Disorder in Motor Vehicle Crash Survivors

Acute stress disorder (ASD) and posttraumatic stress disorder (PTSD) may develop after exposure to events, such as motor vehicle crashes (MVCs), that are experienced as intensely distressing or threatening. Research suggests that intense physical or psychological trauma can cause long-standing alterations in the neurophysiological responses to stress and contribute to the development of health problems (Chung, Dennis, Easthope, Werrett, & Farmer, 2005; Nutt, 2000). Individuals may endure a number of unpleasant symptoms, such as hyperarousal symptoms (hypervigilance, irritability, sleep disturbances, increased startle, increased heart rate), that are associated with these alterations. Evidence is emerging that dysregulation of the sympathetic-adrenal medullary/autonomic (SAM) and hypothalamic-pituitary-adrenal axis (HPA) stress response systems may underlie some of these troublesome symptoms. Previous studies have primarily been retrospective or cross-sectional and focused on populations with chronic trauma exposure or chronic stress disorders, thereby limiting the potential to assess relationships between emerging stress symptoms and neurobiological parameters. The purpose of present study is to examine the linkages among emerging acute stress symptoms, neurobiological correlates, and progression to PTSD in MVC survivors.

MVCs are among the most frequent traumatic events culminating in PTSD (Butler & Moffic, 1999; Silove, Brooks, Steel, Blaszczynski, Hillman, & Tyndall, 2006). In a study of survivors of MVCs, Ursano and colleagues (1999) found high rates of PTSD. One month after a MVC, 34.4% of survivors met criteria for PTSD. At 3 months 25.2% and at 6 months 18.2% of survivors met criteria for PTSD. Harvey and Bryant (1999a) found that approximately 13% of MVC survivors were sufficiently traumatized by the event to meet current criteria for ASD and that another 21% had subsyndromal ASD. Approximately 80% of those who met full criteria for ASD subsequently developed PTSD (Harvey & Bryant, 1999c). Although risk estimates for PTSD subsequent to MVCs vary among these and other studies, the rates remain high overall and support a research focus directed towards early identification and targeted therapies for this population. Although current interventions such as cognitive behavioral therapy, selective serotonin reuptake inhibitors, and other medications may help to relieve symptoms and attenuate the course of PTSD for many sufferers of traumatic stress-related symptoms, they may not provide adequate relief for all (Baldwin et al., 2005; Boehnlein & Kinzie, 2007; Karl, Malta, Alexander, & Blanchard, 2004).

Hyperarousal in the acute post trauma phase may be predictive of long-term PTSD (Harvey & Bryant, 1999a; Marshall, Schell, Glynn, & Shetty, 2006; McFarlane, 2000; Shalev et al., 1998). Physiological hyperarousal is the most frequently experienced of the three symptom clusters in ASD or PTSD. Harvey and Bryant (1999b) found that 75% of people experience hyperarousal symptoms in the first month following a MVC. Environmental cues… sounds, smells, news reports, activities … may serve as reminders of the traumatic event, triggering autonomic, endocrine, and behavioral responses, including fear, anxiety and physiological arousal (Ledoux & Gorman, 2001). In traumatic events such as MVCs, cues can be pervasive. King (1988) found that

tangible reminders of a traumatic event limited the use of denial as a coping strategy. In MVC survivors, injuries that result in scars, changes in mobility or persistent pain or damage to property may act as tangible reminders and decrease ability to cope by denial or avoidance. The extent of injury suffered may also affect coping (Blanchard & Hickling, 1997). Indeed, there is some evidence that type of coping strategies used post-trauma may help to predict the development of PTSD (Jeavons, 2000). Also, information about coping may be useful in the interpretation of trauma symptom severity data.

The present study used an integrative, multidimensional prospective approach to assess whether physiological hyperarousal in the immediate aftermath of a MVC is associated with the subsequent development of PTSD. The contribution that choice of coping strategies may make to hyperarousal symptom severity and the development of ASD and PTSD was also assessed. In addition, the study examined the potential relationship between the SAM stress system, as measured by acoustic startle and heart rate, and the HPA system, as measured by salivary cortisol levels. The present study is innovative in that it included measurement of both salivary cortisol levels and acoustic startle responses in the same subjects.

Method

Hypotheses

Primary Hypothesis:

Individuals who demonstrate higher levels of hyperarousal, as measured by: (a) higher symptom frequency scores on the revised Impact of Event Scale (*IES*; e.g., more frequent sleep disturbance, irritability, vigilance and impaired concentration); (b) greater startle amplitude; and (c) increased heart rate, at the initial measurement session will be more likely than those who show low hyperarousal to meet criteria for PTSD at the second measurement session.

Secondary Hypotheses:

1. Individuals with ASD and no history of prior trauma will show: (a) increased heart rate; (b) exaggerated startle; (c) normal startle habituation.
2. Individuals with PTSD will show evidence of: (a) increased heart rate; (b) exaggerated startle; (c) slower habituation to startle.
3. Severity of IES hyperarousal symptoms will be related to: (a) startle amplitude; (b) heart rate; (c) cortisol levels; (d) choice of coping strategies; (e) perceived coping effectiveness; (f) perceived stress level.

Design

A repeated-measures, correlational design was used. Subjects were assessed two weeks post-MVC (referred to as "session 1") and again six weeks post-MVC (referred to as "session 2"), to enable within-subject and between-subject comparisons by group and time. A combination of self-administered and interviewer-administered questionnaires was used to measure psychological variables. Visual analogue scales were used to assess perceived stress, perceived coping effectiveness, and pain. Physiological measures included heart rate, acoustic startle, and salivary cortisol levels. For the purposes of analyses, the sample was subdivided according whether they met criteria for ASD and/or PTSD and hyperarousal level. Correlational statistics were used to identify related variables. One-way ANOVA was used for within-group and between-group comparisons. Paired samples *t*-tests were used for within-subject comparisons over time. Multiple regressions were used to identify factor contributions.

Recruitment Protocol

Participants were recruited through the emergency departments (ED) of two urban tertiary care centres and one urban primary care centre. ED staff who volunteered to recruit participants were informed orally and in writing about the study and the inclusion and exclusion criteria. All participants who gave written permission to the ED recruiters to be contacted about the study by the investigator were contacted by telephone about one week after the crash. When contacted, those who indicated willingness received detailed explanations of the purpose and design of the study and were invited to ask questions. They were advised that coming to the unit did not imply consent and that they were free to withdraw from participating in the study at anytime without explanation and without bias or judgment from the investigator. They were also advised that there were eligibility criteria for participation in the study and invited to answer a series of screening questions designed to identify in advance those who were not eligible; this was intended to save ineligible participants the time and inconvenience of coming to the research unit. Those who indicated interest in participation were given appointments to come to the research unit for private individual interviews and assessments. When potential participants arrived at the unit for their initial appointments, they were informed orally and in writing about the proposed study and their rights as subjects (Appendix A). The equipment that was used to assess startle and heart rate was shown and explained. Written consent was then obtained from those who volunteered to participate (Appendix B).

Measurement of Psychological Variables

A combination of self-administered and interviewer-administered questionnaires was used to measure psychological variables. The *Acute Stress Disorder Interview scale (ASDI:* Bryant, Harvey, Dang, & Sackville, 1998) was administered in session 1 to

identify participants who met DSM-IV diagnostic criteria for ASD. It contains 19 dichotomously scored items and provides a total score (1 to 19) of ASD severity. To meet criteria for ASD, participants must experience symptoms from three clusters – re-experiencing, avoidance, and hyperarousal – plus dissociation. Individuals who do not experience dissociative symptoms have been classified as "subsyndromal ASD" (Harvey & Bryant,1999b).

The *Life Events Checklist* (page 1 of the *Clinician-Administered PTSD Scale-I [CAPS-I]*), was used in session 1 to assess previous exposure to traumatic events. The full CAPS-I was used in session 2 to identify participants who met criteria for PTSD (scored as either "yes" or "no") and to assess total PTSD symptom frequency (likert scales: 0 = never; 4 = daily or almost daily) and intensity (0 = none to 4 = extreme) for each of the three CAPS-I symptom clusters – re-experiencing, avoidance, and hyperarousal. Meeting criteria for PTSD according to the CAPS-I requires that subjects experience at least one re-experiencing symptom, three avoidance symptoms, and two hyperarousal symptoms. The revised *IES* was used in both sessions to evaluate longitudinally the three main symptom clusters of the DSM-IV for ASD and PTSD: hyperarousal, intrusion, and avoidance (Horowitz, M., Wilner, N., & Alvarez, W., 1979). IES items were scored according to severity on a 0 (not at all) to 4 (extremely) likert scale. The *Mini International Neuropsychiatric Interview (MINI;* Sheehan et al., 1997) was administered in session 1 to identify Axis I psychiatric disorders that may have excluded participation and in session 2 to assess emerging comorbidity. The short 62-item *Mood and Anxiety Symptom Questionnaire (MASQ)* was used in both sessions to assess symptom severity (likert scale: 1 = not at all; 5 = extremely) in individuals with comorbid depression (Clark & Watson, 1991). The situational version of the 28-item *COPE Inventory* (Carver, 1997) was used to assess coping with the MVC event in

particular. Items were scored according to frequency of use (likert scale: 1 = not at all; 4= doing this a lot), then pairs of items were totaled to derive a total score for the 14 subscales. All 28 items were totaled to determine a "total COPE score" (minimum score = 28; maximum score = 112).

Physiological data (cortisol, startle, heart rate) were collected before the questionnaires were presented. To assess the likelihood of physiological data being influenced by differences in stress levels, analogue scales were presented immediately before cortisol and startle were assessed. Participants marked a point on visual analogue scales designed for the study to indicate current perceived stress level (1 = least ever experienced to 10 = most ever experienced), current pain level (1 = none at all to 10 = worst possible), and coping during the preceding 24 hours (1 = very poorly to 10 = very well). Questionnaires were presented in the following order: demographic questionnaire (session 1 only), ASDI (session 1 only), MINI (session 1 only), COPE, MASQ, IES, CAPS-I (session 2; only pg. 1 at session 1).

Neurophysiological Measures

Neurophysiologic measures used were heart rate (HR), salivary cortisol, and acoustic startle. A fingertip sensor and Psylab computerized monitoring equipment was used to record HR during startle assessment.

Salivary Cortisol Protocol

Salivary cortisol samples were collected in Salivettes© (Sarstedt Inc.) pre- and post-startle assessment and at home 30 minutes after awakening the morning following the first session and the morning preceding the second session. The collection protocol used is described by King and Hegadoren (2002). At-home samples were returned in prepaid Xpresspost© mailers. Samples were centrifuged, aliquoted and stored at –80C until analyzed. ELISA kits manufactured by Immuno Biological Laboratories, Hamburg,

Germany and distributed by Research Diagnostics Inc for in vitro research use were used to process the salivary cortisol samples.

Startle Protocol

A commercial computerized acoustic startle response (ASR) system (San Diego Instruments Inc., San Diego, CA, USA) with an infrared eyeblink registration module was used to measure the startle response. The system delivers binaural acoustic stimuli via headphones. The ASR signal was filtered at 1000 hertz. Headphones were placed in alignment with the auditory canal and amplifier gain settings were held constant to promote consistency in acoustic signal intensity across participants and test sessions. To enable noninvasive, simultaneous bilateral measurement of the eyeblink response, subjects wore safety eyeglasses with lenses that are fitted bilaterally with infrared transceivers. An infrared beam directed onto the cornea is reflected back to the sensor when the eye blinks. Thus, the system measured reflectance rather electromyography. Participants sat in a recliner chair with their eyes open and looking straight ahead. The assessment room temperature and lighting were constant. Acoustic stimuli were bursts of white noise (40 milliseconds [ms] duration, 100 decibels [dB] intensity) with instantaneous onset over continuous background white noise (70dB) through headphones binaurally. The intensities of the acoustic stimuli and the white noise were similar to other studies (Shlik, Zhou, Koszycki, Vaccarino, & Bradwejn, 1999; Light & Braff, 2001). Since the present study included pulse alone and prepulse trials, the protocol for stimulus delivery described by Cadenhead, Swerdlow, Shafer, Diaz, and Braff (2000) was used. The protocol was replicated as described by Cadenhead et al., except that the maximum stimulus intensity used was 100 dB rather than 115 dB. Also, the prepulse trials were in accordance with the recent modification of the prepulse component of the protocol (Cadenhead & Schafer, 2002, personal communication).

Each startle session began with a five minute acclimation period for participants prior to testing. A 70-dB background noise was delivered and continued constantly throughout the acclimation period and test session. The startle protocol used three 'trial' types: pulse alone, prepulse followed by pulse, and no stimulus. A pulse (a 100 dB burst of white noise for duration of 40 ms) was presented either alone or following a prepulse (an 86 dB burst of white noise for duration of 20 ms). When used, a prepulse preceded a pulse by 30 ms, 60 ms, or 120 ms interstimulus intervals [ISI] in pseudorandom order. Intertrial intervals ranged from 8-22 seconds in pseudorandom order.

A startle test session was comprised of four consecutive blocks of trials. The first and last blocks each consisted of 5 pulse alone trials. The second and third blocks each included prepulse trial types (6 trials each of 30, 60, 120 ms ISI) that were sequenced and intermixed with pulse alone (12 trials) and no stimulus (12 trials) trial types in a set pseudorandom pattern. A complete startle session was 58 trials (116 eyeblink responses assessed per subject). The total time for a startle session was 25 minutes.

Although evidence of a robust circadian pattern of acoustic startle was reported in rats (Chabot & Taylor, 1992; Frankland & Ralph, 1995), it is not known whether there may be a circadian rhythm of startle in humans. Exogenous corticosteroid was found to influence startle in a study of healthy volunteers (Buchanan, Brechtel, Sollerslli, & Lovallo, 2001); however, it is not known whether circadian fluctuations in endogenous cortisol levels are sufficient to influence startle. Subsequently, the present study assessed startle at the same time of day for both phases for each subject to control for potential circadian effects and those on exogenous corticosteroids were excluded.

Digital ASR-eyeblink signals were full-wave rectified and smoothed by an averaging routine set to calculate a rolling average (smooth) of 10 data points. The system was set to record at a rate of 1000 samples per second starting 200 ms before

each pulse or prepulse stimulus. The 200 ms pre-recording permitted identification, during trial-by-trial visual inspection of right- then left-eyeblinks, of any extraneous voluntary or spontaneous eyeblinks (defined as baseline shift greater than 35% of maximum in the absence of a stimulus or when onset and peak latencies differ by > 95 ms in stimulated responses) that may contaminate ensuing stimulus responses. Fewer than 5% of trials were subsequently excluded on this basis. Startle data scoring was both computer-assisted (EMG Data Reduction Software, San Diego Instruments Inc., San Diego, CA, USA ,2003) and manual in accordance with response quantification methodology described by Blumenthal et al. (2005, p. 10). Consistent with the recommendations of Blumenthal et al., all trials for all subjects were scored by one rater to avoid inter-rater variability. To increase reliability, every trial was scored twice, independently of previous scoring, by the same rater. Trials with the baseline shift greater than 20 percent of maximum were rejected by the software (recommended default setting, San Diego Instruments Inc.). In cases where an eyeblink waveform showed multiple peaks, the maximum value was identified as the peak.

Data Analyses

The independent variables were hyperarousal, ASD, and PTSD. The dependent variables analyzed were: perceived stress, perceived coping efficacy, perceived pain severity, heart rate (HR), salivary cortisol, startle amplitude, habituation, peak latency, latency facilitation, and PPI. Microsoft Excel, SPSS 10.0.7, and EMG Data Reduction Software were used for data analysis. Raw data were coded and entered in accordance with established protocols for software and standardized instruments. Descriptive statistics were used to describe the demographic characteristics of the sample and extraneous variables. Left and right startle eyeblink data were averaged for each trial type within each block. In accordance with the startle protocol, five participants who

exhibited a relative lack of startle reactivity (the startle eyeglasses fit these participants poorly), resulting in an excessive number of rejected trials, at one startle session each were considered "non-responders" and excluded from startle phase analyses. This is a lower non-responder rate than the 25% to 31% rate that may be considered typical (Cadenhead et al., 2000). Startle amplitude (mV) was determined by averaging startle responses. Habituation was quantified by calculating the arithmetic difference of amplitude in mV for pulse alone trials in blocks 1 and 4 (pulse alone block 1 – pulse alone block 4). The time required to generate the peak of the first amplitude waveform, in which the baseline value shifted at least 10% above baseline, was used to determine peak latency (ms) for each pulse alone trial. Peak latencies were then averaged for each block of pulse alone trials. The mean of pulse alone trials minus the mean of prepulse trials was used to quantify latency facilitation (defined as the reduction in latency in ms by a prepulse). PPI was computed using difference scores: arithmetic difference between mean of pulse alone trials and mean of each of the prepulse conditions [(pulse alone) – (prepulse + pulse)]. For comparative purposes, PPI was also computed using percentage scores: arithmetic difference between mean of pulse alone trials and mean of each of the prepulse conditions divided by the mean of pulse alone trials multiplied by 100 [(pulse alone) – (prepulse + pulse)]/(pulse alone)*100]. Although percentage scores correct for individual variability, difference scores have been considered the best way to report PPI (Cadenhead et al.).

For the purposes of comparative analyses, the sample was subdivided according to hyperarousal level and whether they met criteria for ASD or PTSD. Because dissociation has been shown not to add predictive power for the development of PTSD to the overall diagnosis of ASD (Brewin, Andrews, Rose, & Kirk, 1999; Harvey & Bryant, 1999c), for purposes of analyses in this study the four subjects who met all criteria for

ASD except for dissociation (i.e., were subsyndromal) were included in the "ASD"/"ASD criteria met" group.

Correlational statistics were used to identify related variables and determine direction of relationships. One-way ANOVA and *t*-tests were used for within-subject and between-subject comparisons. Repeated measures ANOVA were used to compare groups across sessions. An α of 0.05 was used for all analyses. The results are described in the following section.

Results

Statistical analyses show varying sample sizes, since data were missing for some assessment components for a few subjects. Two of the 26 participants who completed the initial assessment but were unable to complete the second assessment were subsequently excluded from longitudinal comparisons. Baseline cortisol samples were missing for three participants at session 1 and two participants at session 2 (two samples were not returned and three yielded insufficient quantities for analyses). In addition, blocks of startle trials with no stimulated eyeblinks were excluded from analyses.

Sample Characteristics

Twenty-six MVC survivors [mean age = 29.4 years], 13 males and 13 females, signed informed consent forms approved by the institutional ethics review board. All were drivers (n = 20), passengers (n = 5), or a pedestrian struck (n = 1) in the MVC. Most were alone (n = 16) or with one other person (n = 8) or pet (n = 1) in the vehicle. All but two of the crashes involved another vehicle. All of the crashes occurred in daylight hours. According to triage records and self-report alcohol was not a factor in any of the crashes; however, blood alcohol levels were not assessed. Triage scores on the *Canadian Triage and Acuity Scale (CTAS)* ranged from '2 = emergency' (n = 3) to '5 =

non-urgent' (n = 1). The majority were rated '3 = urgent' (n = 10) or '4 = less urgent' (n = 12). Physical injuries included soft tissue (n = 16), lacerations requiring sutures (n = 3), fractures (n = 1) and none or minor (n = 6). None sustained head injuries that resulted in loss of consciousness.

All participants completed the initial assessment (referred to as "session 1") between fourteen and sixteen days post-MVC. Twenty-four were retested four weeks after their initial assessment (referred to as "session 2") at the same time of day as in their initial assessment. Two of the 26 (both males and both with the most urgent triage scores and injury severity scores of the sample) did not complete session 2 (one became ineligible due to commencement of antidepressant medication therapy and one dropped out due to MVC-related symptoms that precluded travel to the research unit) and were excluded from longitudinal comparisons.

All participants spoke and read English. All except seven had post-secondary education. None ingested caffeinated beverages or smoked cigarettes within 2 hours of testing. None had ingested analgesics within four hours of testing. Six females used oral contraceptives and one used hormonal replacement therapy. None of the participants used any other prescription or non-prescription drugs with relevant psychotropic properties, including antidepressants, herbal products, street drugs, or alcohol, on a regular basis or within the 24 hour period preceding testing. None received systemic corticosteroid therapy during the preceding 3 months. None of the participants were pregnant or breast-feeding. Only six participants consumed more than 3 cups of caffeinated beverage on a daily basis and six smoked cigarettes. Three reported a history of an episode of depression but none were treated for depression. None had histories of other DSM-IV Axis I psychiatric disorders [as determined by the *MINI*] active medical or neurological condition, previous serious medical or surgical condition, or self-

reported hearing deficit [deafness or inability to hear sounds in range of 45 to 114 decibels in either ear].

Psychosocial Outcomes

Acute Stress Disorder

Of the total sample of MVC survivors, 62% were in the ASD group (12 met full criteria for ASD and 4 were subsyndromal) and 38% were in the non-ASD group (did not meet criteria for ASD). The ASD (n = 16) and non-ASD (n = 10) groups were closely matched in terms of triage score, type of injuries, trauma centre accessed, and role in the accident (driver or passenger).

The ASD group showed significantly higher perceived stress level ($F = 10.66$, $df = 1,25$, $p = 0.003$), higher perceived pain level ($F = 4.68$, $df = 1,25$,$p = 0.041$), and lower perceived coping efficacy ($F = 4.60$, $df = 1,25$,$p = 0.042$). As shown in Table 5.1, ASD was also associated with higher total coping scores and coping by denial, self-blame, and self-distraction at session 1. ASD was associated with higher total coping scores and with coping by self-distraction, active coping, emotional support, instrumental support, and venting at session 2. The ASD group also showed significantly greater symptoms on all IES subscales (range: $F = 11.96$, $df = 1, 23$, $p = 0.002$ to $F = 24.25$, $df = 1, 23$, $p = 0.000$) and more severe anxiety symptoms on all MASQ subscales ($p < 0.05$) at both sessions, except for anhedonic depression ($p = 0.24$) at session 1.

Table 5.1

ANOVA: Differences in Total COPE Score and COPE Subscale Scores by ASD Group (ASD or Non-ASD)

COPE scale	*df*	*F*	*p*
Session 1			
Total coping score	1, 25	5.90*	0.023
Denial	1, 25	4.35*	0.048
Self-blame	1, 25	4.39*	0.047
Self-distraction	1, 25	4.31*	0.049
Session 2			
Total coping score	1, 23	11.04*	0.003
Self-distraction	1, 23	6.37*	0.019
Active coping	1, 23	5.00*	0.036
Emotional support	1, 23	7.42*	0.012
Instrumental support	1, 23	11.14*	0.003
Venting	1, 23	9.47*	0.006

*$p < 0.05$, **$p < 0.01$

Posttraumatic Stress Disorder

PTSD as measured by the CAPS-I was highly correlated with PTSD as measured by the MINI ($r = 0.92$, $p = 0.000$). Of the 12 participants who met CAPS-I criteria for PTSD, all but one first met criteria for ASD (n = 11). Highly predictive of the later development of PTSD, using repeated measures ANOVA, were ASD criteria met ($F = 18.53$, $df = 1, 23$, $p = 0.000$), ASDI total score ($F = 41.5$, $df = 1, 23$, $p = 0.000$), ASDI

hyperarousal score criterion E (F = 19.7, df = 1, 23, p = 0.000), and IES hyperarousal (F = 48.7, df = 1, 23, p = 0.000) in the early aftermath of the MVC. As shown in Table 5.2 and Figure 5.1, the ASD group showed significantly greater symptom frequency and intensity on all PTSD (CAPS-I) symptom clusters.

Table 5.2

ANOVA: Differences in PTSD (CAPS-I) Symptom Clusters by ASD Group (ASD or Non-ASD)

PTSD (CAPS-I) Symptom Cluster	*df*	*F*	*p*
Reexperiencing symptoms			
Frequency	1, 23	12.77**	0.002
Intensity	1, 23	16.26***	0.001
Avoidance sympoms			
Frequency	1, 23	11.54**	0.003
Intensity	1, 23	12.89**	0.002
Hyperarousal symptoms			
Frequency	1, 23	18.53***	0.000
Intensity	1, 23	20.04***	0.000

*p < 0.05, **p < 0.01, ***p < 0.001

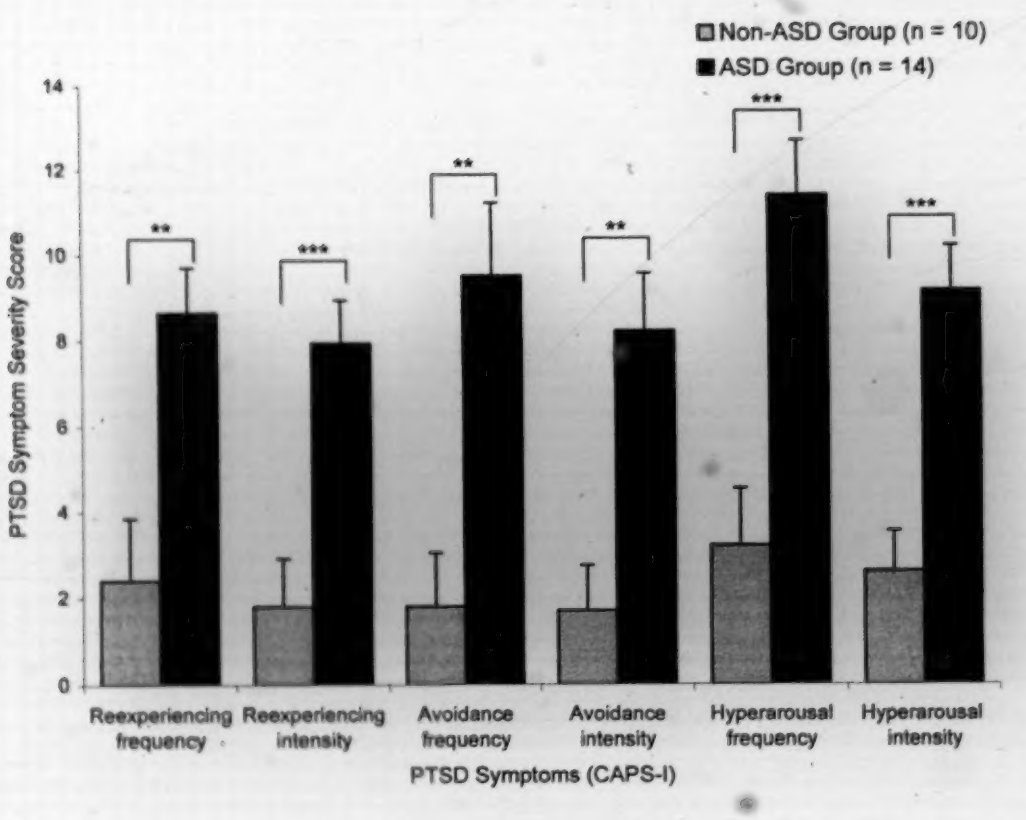

Figure 5.1. Relationship between ASD at baseline and PTSD symptom frequency and intensity. $^{**}p < 0.01$, $^{***}p < 0.001$.

Dissociation. Baseline recollections of peritraumatic dissociation (ASDI) were also highly predictive of follow-up PTSD ($F = 17.6$, $df = 1, 23$, $p = 0.000$). As shown in Figure 5.2, dissociation was associated with increased severity for all symptom clusters -- reexperiencing (frequency: $F = 6.16$, $df = 1,23$, $p = 0.021$; intensity: $F = 12.5$, $df = 1,23$, $p = 0.002$; avoidance (frequency: $F = 4.676$, $df = 1,23$, $p = 0.042$; intensity: $F = 4.45$, $df = 1,23$, $p = 0.047$); hyperarousal (frequency: $F = 17.49$, $df = 1,23$, $p = 0.000$; intensity: $F = 16.98$, $df = 1,23$, $p = 0.000$).

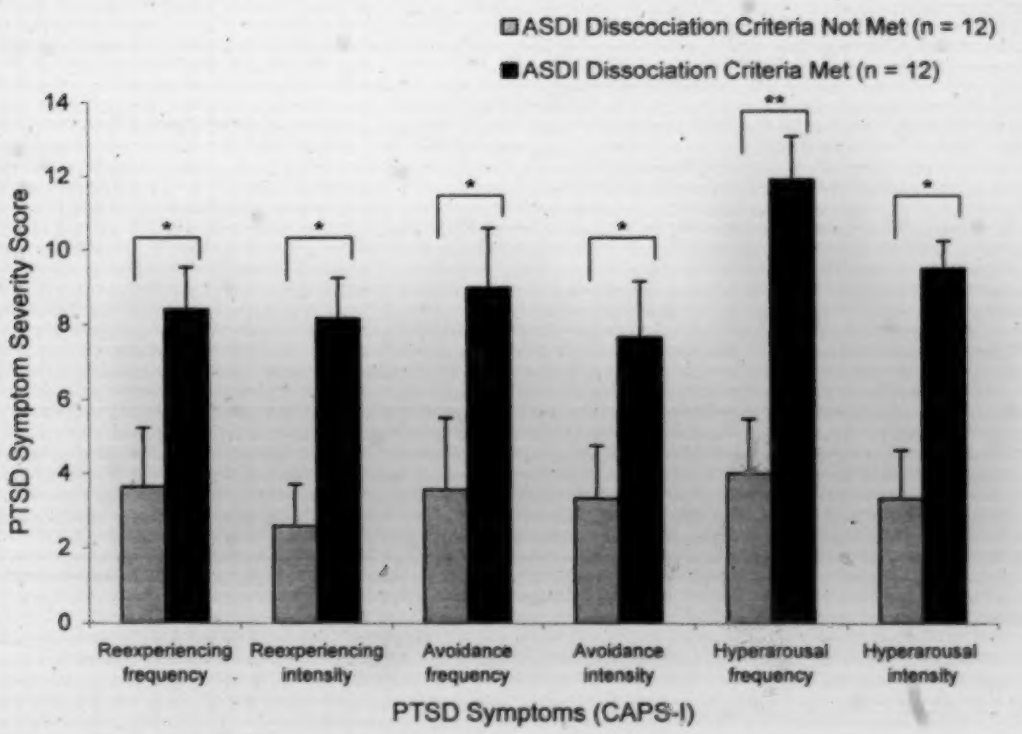

Figure 5.2. Relationship between peritraumatic dissociation (ASDI) at baseline and PTSD symptom severity. $^{*}p < 0.05$, $^{**}p < 0.01$.

Other dependent variables. PTSD was highly correlated with all MASQ subscales at session 2 (range: $r = 0.55$, $p = 0.005$ to $r = 0.76$, $p = 0.000$). PTSD was correlated ($p < 0.05$) with all IES subscales at both session 1 and 2. Perceived stress level at session 1 (mean = 5.2 out of 10; $SD = 2.31$) was highly and positively correlated with PTSD ($p = 0.028$) and all PTSD subscales (range: $p = 0.028$ to $p = 0.001$). PTSD was associated with higher total COPE scores ($p = 0.012$) and more frequent use of disengagement, self-distraction, substance abuse, and instrumental support coping strategies (range: $p = 0.032$ to $p = 0.003$).

Extraneous variables. PTSD was not correlated with any of the extraneous variables. The nature of the MVC event was not associated with PTSD. The PTSD and non-PTSD groups were closely matched according to triage score, type of injuries sustained in the crash, which of three emergency departments were accessed, whether they were drivers or passengers, whether they were alone or with others in the vehicle, and whether the crash involved another occupied vehicle.

Hyperarousal

All MVC survivors had some hyperarousal symptoms (as measured by the ASDI and IES). A paired sample differences *t*-test showed that MVC survivors (n=24) experienced significantly greater ($t = 3.12$, $df = 23$, $p = 0.005$) hyperarousal symptoms (IES) at session 1 (mean = 11.19; $SD = 7.30$) than session 2 (mean = 8.17; $SD = 7.25$).

Subgroups. Participants were divided into low hyperarousal (LH) and high hyperarousal (HH) groups based on IES hyperarousal score at session 1 (LH score range: 2 to 9, $M = 4.7$, $SD = 2.46$, $n = 13$; HH score range: 12 to 24, $M = 17.7$, $SD = 3.66$, $n = 13$) and at session 2 (LH score range: 0 to 8, M 2.85, $SD = 2.74$, $n = 14$; HH score range: 10 to 23, $M = 15.6$, $SD = 4.25$, $n = 10$). Hyperarousal levels tended to endure over time, with all but three participants (two moved from HH to LH group and one from LH to HH group by session 2) comprising the same hyperarousal group across both sessions. ANOVA showed no significant differences between the groups on any of the extraneous variables, including age gender, education, social support, caffeine intake, and nature of the MVC event.

Dependent variables. The HH and LH groups differed significantly on several dependent variables as seen in Table 5.3. The HH group had higher perceived stress and higher perceived pain levels at session 1, but not at session 2. At both sessions, HH

Table 5.3

ANOVA: Dependent Variables which showed Significant Differences by Hyperarousal Group (Low or High Hyperarousal)

Variable	*df*	*F*	*p*
Session 1			
Perceived stress level	1,25	11.30**	0.003
Perceived pain level	1,25	6.43*	0.018
Depression attributed to MVC	1,25	5.33*	0.030
COPE: Total coping score	1, 25	4.53*	0.044
Denial	1, 25	8.78**	0.007
Self-blame	1, 25	8.30**	0.008
Disengagement	1, 25	4.91*	0.036
Session 2			
COPE: Total coping score	1, 23	12.27**	0.002
Self-distraction	1, 23	8.35**	0.008
Active coping	1, 23	9.94**	0.005
Emotional support	1, 23	5.27*	0.032
Instrumental support	1, 23	9.47**	0.006
Self-blame	1, 25	6.35*	0.020
Venting	1, 23	9.08**	0.006

*$p < 0.05$, **$p < 0.01$

was associated with higher total coping scores. HH at session 1 was associated with coping by denial, disengagement, and self-blame. HH at session 2 was associated with coping by self-distraction, active coping, venting, self-blame and use of instrumental and emotional support (Table 5.3). HH was also associated with significantly greater MASQ symptoms, except for anhedonic depression, at session 1 and with all MASQ subscales at session 2 (Table 5.4).

Table 5.4

ANOVA: Differences in MASQ Subscales
by Hyperarousal Group (Low or High Hyperarousal)

	Session 1			*Session 2*		
MASQ Symptoms	*df*	*F*	*p*	*df*	*F*	*p*
Anxiety	1, 25	24.42**	0.000	1, 25	33.89***	0.000
Anxious arousal	1, 25	23.86**	0.000	1, 23	23.75**	0.000
Depressive	1, 25	8.04**	0.009	1, 23	7.95*	0.010
Anhedonic	1, 25	3.7	0.066	1, 25	8.15*	0.009

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

PTSD. At both sessions, HH was strongly associated with PTSD (CAPS-I) criteria met (session 1: $F = 121.00$, $df = 1,25$, $p = 0.000$; session 2: $F = 18.53$, $df = 1,23$, $p = 0.000$). HH was also strongly associated with increased symptom frequency and intensity on all PTSD symptom clusters (Table 5.5).

Table 5.5

ANOVA: Differences in PTSD (CAPS-I) Symptom by Hyperarousal Group (Low or High Hyperarousal)

	Session 1			*Session 2*		
PTSD (CAPS-I) Symptom Cluster	*df*	*F*	*p*	*df*	*F*	*p*
Reexperiencing						
Frequency	1, 25	34.83***	0.000	1, 23	15.44***	0.001
Intensity	1, 25	47.84***	0.000	1, 23	20.84***	0.000
Avoidance						
Frequency	1, 25	32.60***	0.000	1, 23	14.33***	0.001
Intensity	1, 25	28.60***	0.000	1, 23	14.02***	0.001
Hyperarousal						
Frequency	1, 25	51.07***	0.000	1, 23	32.72***	0.000
Intensity	1, 25	49.03***	0.000	1, 23	37.26***	0.000

***$p < 0.001$

Comorbid Depression

Of the four participants who met MINI criteria for major depressive disorder (MDD) at session 1, two had histories of depression (both females), one dropped out prior to session 2 due to commencement of antidepressant therapy, and one met criteria for MDD again at session 2. Three subjects, one with a history of depression, were depressed at session 2 but not at session 1.

Gender

Females and males were similarly affected in several symptom clusters. ASD criteria were met by 8 males and 8 females. Dissociation criteria (ASDI) were met by 7 males and 7 females. PTSD (CAPS-I) criteria were met by 6 males and 6 females. At session 1, 7 males and 6 females were in the HH group. At session 2, 4 males and 5 females were in the HH group (2 males with symptoms dropped out before session 2). Four males and 5 females remained in the HH group across sessions.

Of the three participants with a past history of an episode of MDD (diagnosed, untreated, and resolved in all cases more than a year preceding the MVC), two were female. However, the prevalence of depression (MINI) post-MVC was equal or higher in males. At session 1, four participants (2 females with histories and 2 males without histories of depression) met criteria for current depression (MINI). One of the males was unable to participate in session 2 due to commencement of antidepressant therapy. At session 2, four participants met criteria for current depression -- 1 female (with history of depression and met criteria for depression at session 1) and 3 males (1 with history of depression; none met criteria for depression at session 1).

Physiological outcomes

Startle Amplitude and Habituation

ASD group. MVC survivors who met criteria for ASD did not differ from those who did not on any of the startle parameters at session 1, using ANOVA. Although startle amplitude in the first trial block was greater in the ASD than in the non-ASD group (Figure 5.3), this difference in initial startle reactivity was not significant ($F = 1.75$, $df = 1, 21$, $p = 0.202$, $M = 1622$, $SD = 1856$). That startle amplitude decreased between the first and last blocks of startle trials in the ASD group, suggests that some habituation occurred; however, differences were not significant ($F = 2.68$, $df = 1, 18$, $p = 0.120$, $M =$

413, *SD* = 1763). Controlling for effects of prior trauma did not yield significant differences in habituation.

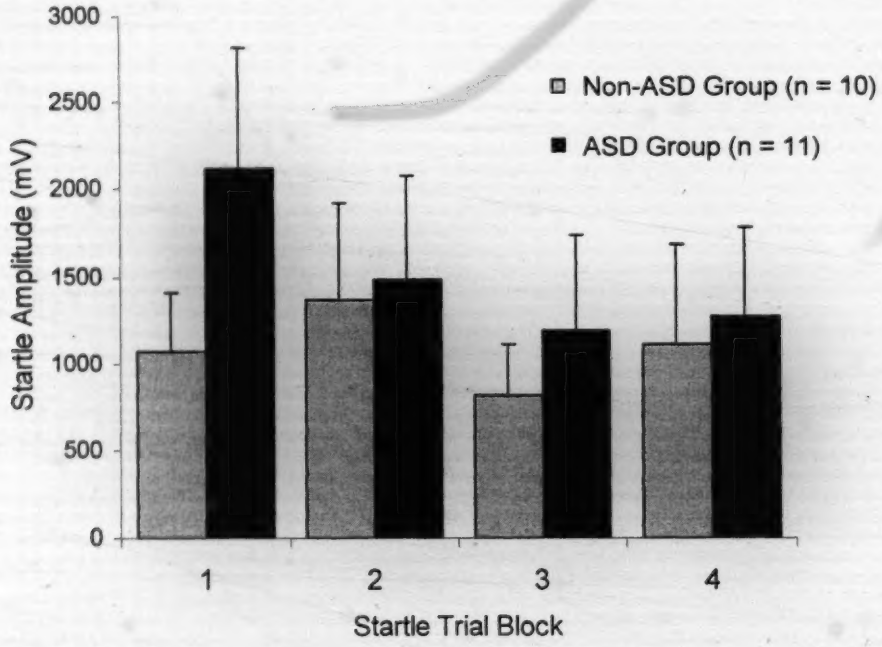

Figure 5.3. Relationship between ASD and startle amplitude and habituation at session 1.

PTSD group. PTSD and non-PTSD groups did not differ on most startle parameters at session 2. Although the PTSD group showed lower startle amplitude at session 2 across all 4 trial blocks (Figure 5.4), these differences were significant only for blocks 2 ($F = 5.08$, $df = 1,19$, $p = 0.037$, $M = 1775.72$, $SD = 2344.71$) and 4 ($F = 5.88$, $df = 1,18$, $p = 0.027$, $M = 1589.18$, $SD = 2353.07$). Differences between groups for block one were not significant ($F = 2.05$, $df = 1, 20$, $p = 0.169$, $M = 1835.87$, $SD = 1995.17$). Habituation in the PTSD group approached significance ($F = 4.21$, $df = 1,18$, $p = 0.056$, $M = 384.205$, $SD = 1121.69$). Controlling for effects of extraneous variables did not yield significant differences in habituation.

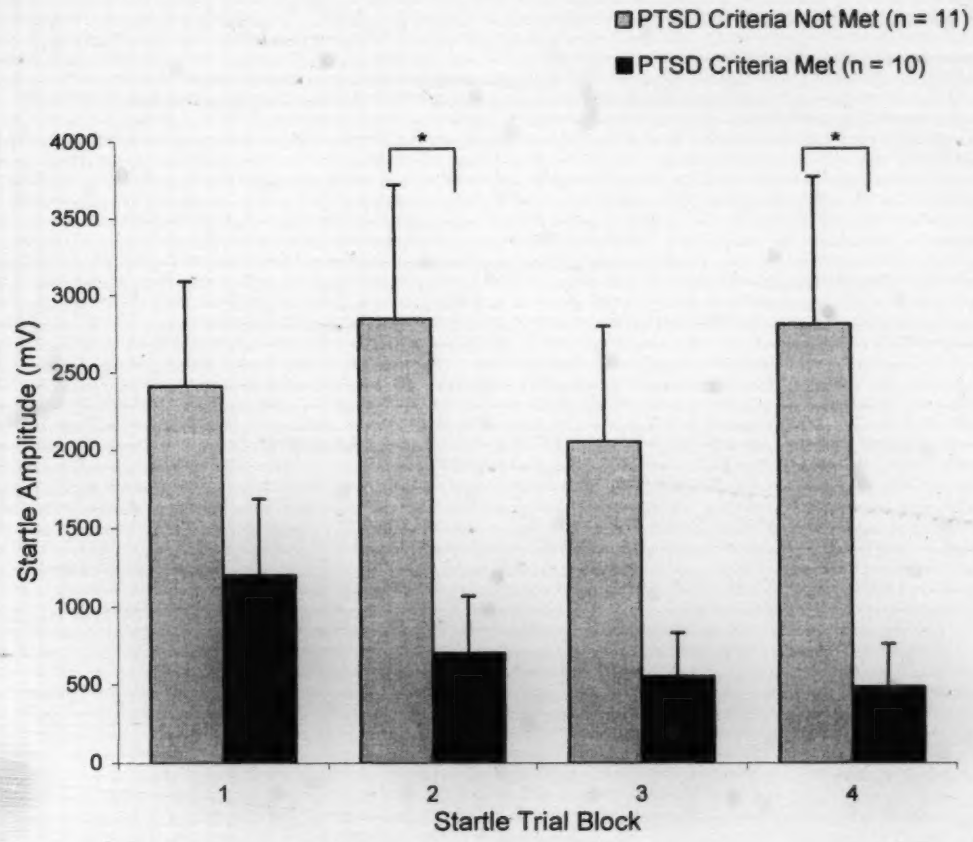

Figure 5.4. Relationship between PTSD and startle amplitude and habituation at session 2. *$p < 0.05$.

Hyperarousal Groups. The highest mean amplitudes for all blocks occurred in the LH group at session 2 (Figure 5.5). Significant differences in amplitude between groups were found in blocks 2 ($F = 4.72$, $df = 1, 19$, $p = 0.043$) and 4 ($F = 5.46$, $df = 1, 18$, $p = 0.032$) at session 2. Although a pattern of progressive decreases in amplitude across trial blocks was observed in the HH group at session 2, habituation was not significant.

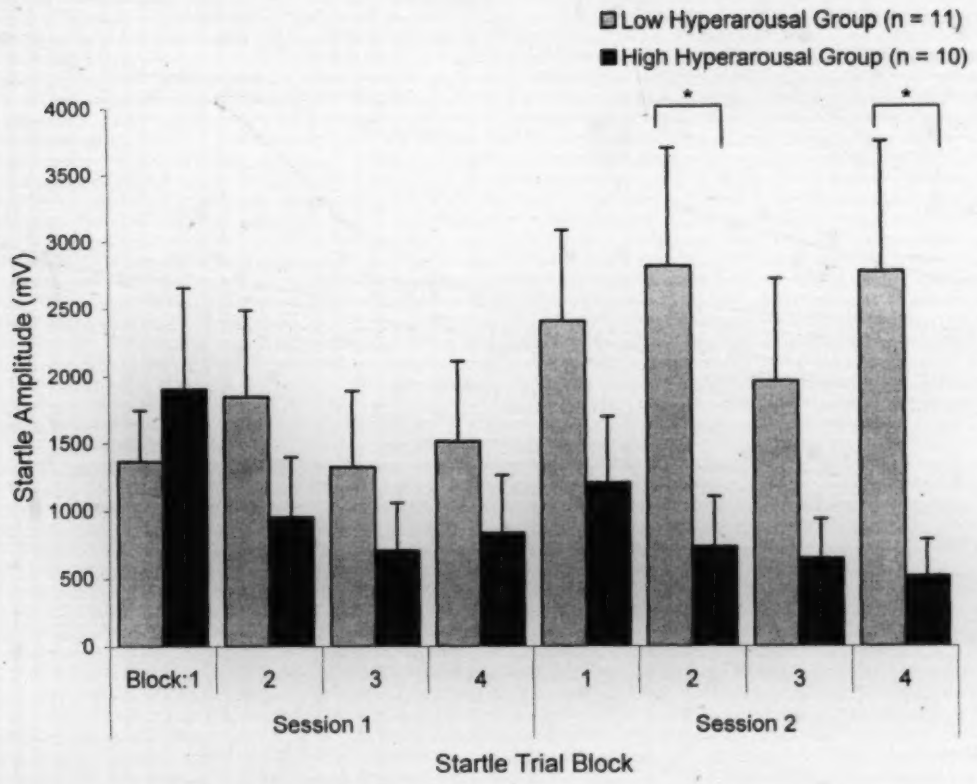

Figure 5.5. Relationship between hyperarousal and startle amplitude and habituation at sessions 1 and 2. $*p < 0.05$.

Prepulse Inhibition

Prepulse inhibition of startle amplitude (sensorimotor gating) was assessed for three prepulse lead intervals: 30, 60, and 120 ms ISI. As shown in Figure 5.6, the ASD group showed higher mean startle amplitude at session 1 in response to prepulses at most lead intervals than did the non-ASD group; these differences were not significant. As shown in Figure 5.7, the PTSD group showed lower mean startle amplitude at session 2 in response to prepulses at all lead intervals than did the non-PTSD group; however, these differences were not significant (range: $F = 0.86$, $df = 1, 19$, $p = 0.37$, $M = 1987$, $SD = 2144$ to $F = 2.25$, $df = 1, 17$, $p = 0.153$, $M = 2260$, $SD = 2285$).

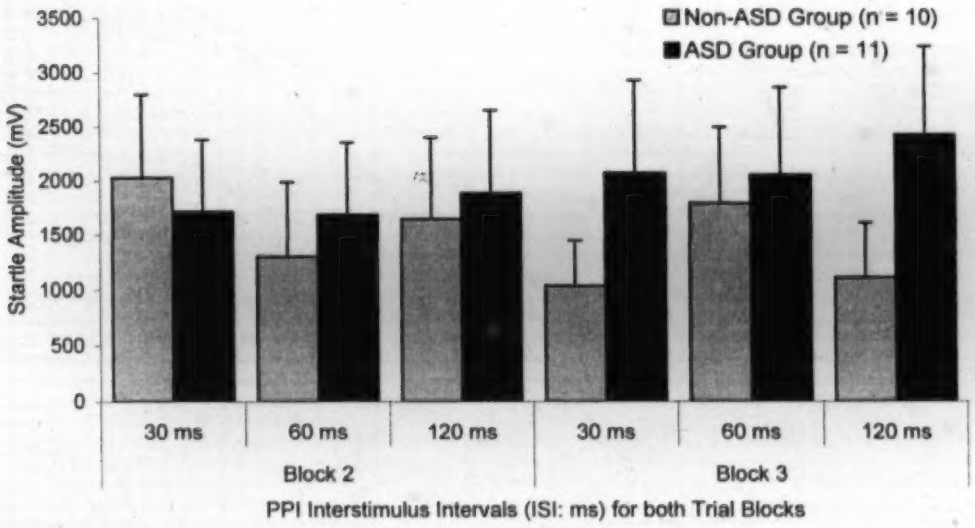

Figure 5.6. Relationship between ASD and startle amplitude for prepulse trials at all lead intervals (ISI) at session 1.

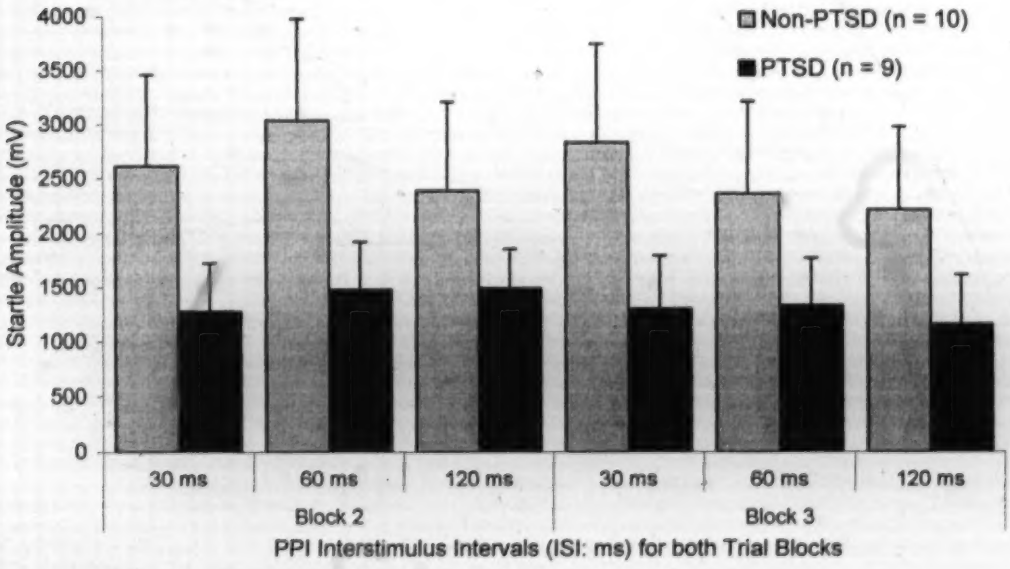

Figure 5.7. Relationship between PTSD and startle amplitude for prepulse trials at all lead intervals (ISI) at session 2.

HH group. Although the HH group demonstrated a greater decrease in startle amplitude in response to each of the 30, 60, and 120 ms ISI conditions than the LH group at session 2 (Figure 5.8), group differences were not significant using either difference scores or percentage reduction scores. Paired samples correlations across sessions were significant (range: $r = 0.504$, $p = 0.033$ to $r = 0.824$, $p = 0.000$) for all ISI and trial blocks, except for 30 ms ISI in block 3. Pulse alone trials that were intermixed with prepulse trials in blocks 2 and 3 are included in Figure 5.8 for comparative purposes.

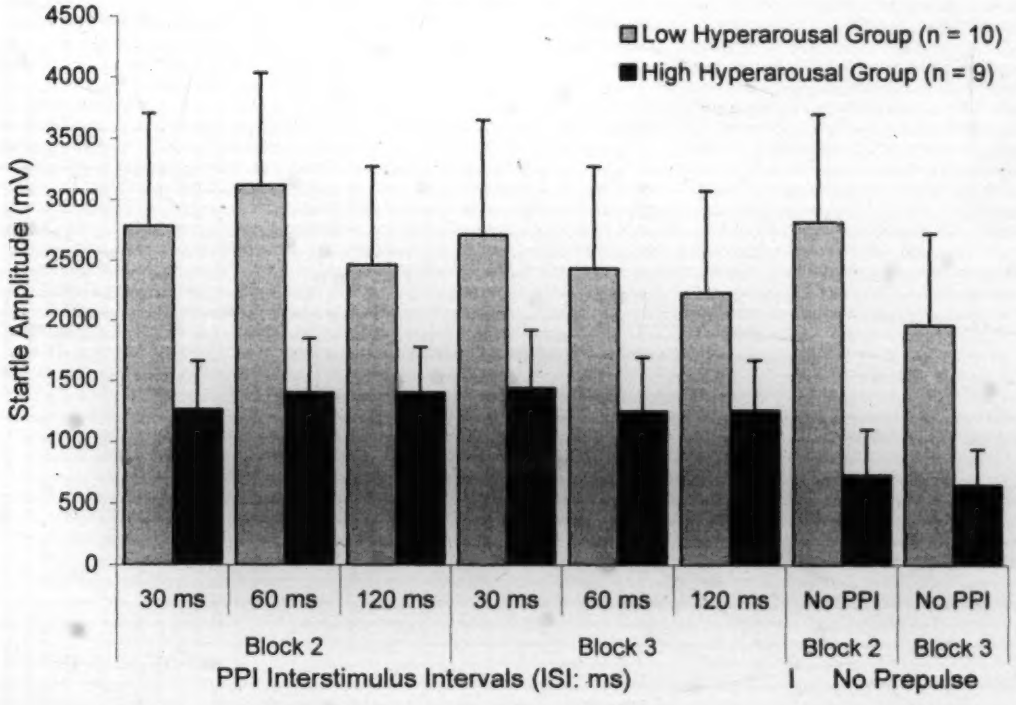

Figure 5.8. Relationship between hyperarousal and startle amplitude for prepulse trials at all lead intervals (ISI) and for pulse alone (no prepulse) trials at session 2.

Heart rate

Heart rate (HR) was monitored at both assessment sessions pre-, during- and post-startle assessment. HR remained highly correlated within participants across and between sessions. ASD, PTSD and hyperarousal were not associated with HR at any time during or across sessions. Controlling for extraneous and dependent variables did not yield significant differences.

Cortisol

Patterns of salivary cortisol levels varied within LH and HH groups (Table 5.6). At session 1, 50% of LH and 36% of HH subjects showed the normal patterns of high baseline (30 minutes after awakening) and lower daytime levels. At session 2, 38% of LH and 50% of HH subjects showed the normal patterns. Several subjects had baseline levels that were close to their daytime levels (referred to as "flat"). A consistent pattern shown at both sessions was that all LH subjects who had low baseline and high daytime cortisol levels did not meet criteria for either ASD or PTSD.

Table 5.6.

Patterns of Salivary Cortisol Levels by Hyperarousal Group (LH and HH)

	Session 1		Session 2	
	LH	HH	LH	HH
Flat Baseline: Daytime	42%	36%	38%	0%
	(n = 5)	(n = 4)	(n = 5)	(n = 0)
Low Baseline: High Daytime	8%	28%	24%	50%
	(n = 1)	(n = 3)	(n = 3)	(n = 3)
High Baseline: Low Daytime	50%	36%	38%	50%
	(n = 6)	(n = 4)	(n = 5)	(n = 3)

HH at session 2 was associated with significantly lower cortisol levels pre- (t = -2.16, df = 15.1, p = 0.047; LH: M = 0.46, SD = 0.43; HH: M = 0.20, SD = 0.10) and post- (t = - 2. 54, df = 16.46, p = 0.022; LH: M = 0.29, SD = 0.20; HH: M = 0.14, SD = 0.06) startle testing at session 1(Figure 5.9). LH and HH groups did not differ significantly in baseline a.m. cortisol levels at either session (session 1 baseline: F = 0.82, df = 1, 22, p = 0.38, M = 0.76, SD = 1.89).

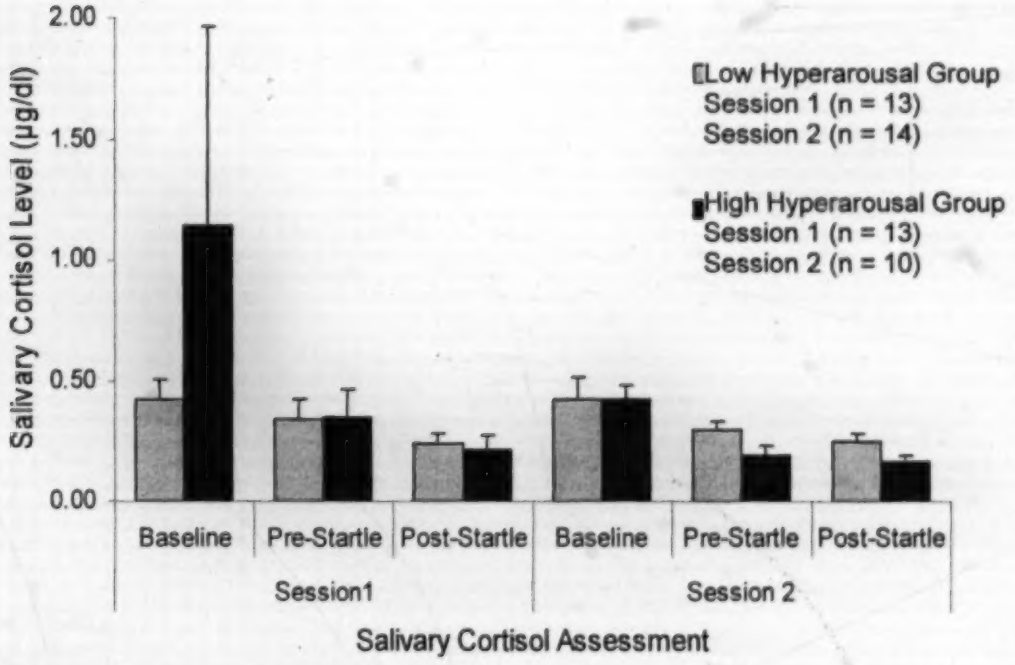

Figure 5.9. Relationship between hyperarousal group and salivary cortisol levels at baseline and pre- and post-startle at sessions 1 and 2.

Other results

When the effects of prior trauma were controlled, startle amplitude and heart rate across the first session were inversely correlated (range: r = -0.4924 to -0.5502, p = 0.045 to 0.022). Increased startle amplitude was associated with decreased heart rate.

Discussion

Of the total sample of MVC survivors, 62% were in the ASD group (the 4 subsyndromal participants were included). Of these, 69% subsequently developed PTSD. These percentages are within ranges reported in other studies (Harvey & Bryant, 1999c; Ursano et al., 1999). Meeting criteria for ASD was associated with increased symptom frequency and intensity on all PTSD and IES subscales, a finding consistent with accumulating evidence that a diagnosis of ASD is highly predictive of subsequent development of PTSD. Although injuries sustained in the MVCs in the present study were non-life threatening, the crash event at the time of occurrence was almost always appraised by participants as a threat to life. Consistent with Schnyder, Moergeli, Klaghofer, and Buddeberg (2001), perceived stress level at two weeks post-MVC was highly and positively correlated with later PTSD and all PTSD subscales. Similar to the finding of Maes, Mylle, Delmeire, and Altamura (2000), trauma events in the year prior to the MVC exerted no effect on incidence or severity of ASD or PTSD. However, in contrast to the finding of Mayou, Bryant, and Ehlers (2001), no differences were found between passengers and drivers on any of the scales, subscales, or physiological measures.

Similar to Freedman et al. (2002), no gender differences were found in the prevalence of ASD, PTSD or PTSD symptom severity. This finding contrasts with studies that reported increased incidence of PTSD in females (Anisman, Griffiths, Matheson, Ravindran, & Merali, 2001; Ursano et al.,1999). Although gender differences are an inconsistent finding among studies, gender differences generally do not appear to influence the prevalence of PTSD beyond one year following acute trauma. The high correlation between PTSD as measured by the CAPS-I and PTSD as measured by the MINI ($r = 0.92$, $p = 0.000$) supports validity of these instruments.

Hyperarousal symptoms, as measured by the IES, were the most frequently experienced of the three symptom clusters that must be present for a diagnosis of ASD or PTSD. Consistent with the primary hypothesis of this study, high hyperarousal levels two weeks post-MVC were strongly predictive of PTSD at six weeks post-MVC. Although hyperarousal symptoms were experienced by all MVC survivors, those with high hyperarousal levels two weeks post-MVC had significantly increased symptom frequency and intensity on all PTSD subscales. These findings are consistent with those reported in other studies (Frommberger, Stieglitz, Nyberg, Schlickewei, Kuner, & Berger, 1998; Harvey & Bryant, 1999a, 1999b; Marshall et al., 2006; McFarlane, 2000; Shalev et al., 1998; Silove, Brooks, Steel, Blaszczynski, Hillman, & Tyndall, 2006).

High hyperarousal levels two weeks post-MVC were associated with significantly greater anxiety symptoms (MASQ), other than anhedonia, and with perceived stress, perceived pain level, depression symptoms that were attributed to the MVC, and total coping scores. High hyperarousal levels six weeks post-MVC were associated with significantly greater anxiety symptoms (MASQ), including anhedonia, as well as higher total coping scores. Passive coping strategies -- denial, disengagement, and self-blame -- were used significantly more frequently in the HH group at two weeks post-MVC. At six weeks post-MVC, the HH group used a greater variety of coping strategies that included some active strategies -- self-distraction, active coping, venting, self-blame, instrumental support and emotional support. The prevalent use of self-blame may be related to the view of MVCs as preventable "manmade" events (Butler & Moffic, 1999). Other researchers also found that active coping strategies tended to be used more over time (Brom, Kleber, & Hofman, 1993; Jeavons, 2000). The finding of increased use of venting over time in the HH group may be adaptive in view of recent findings that debriefing soon after trauma may not be helpful and perhaps even harmful to some (Ballenger,

2000; Bursztajn, Joshi, Sutherland, & Tomb,1999). Although there is evidence that people may begin to self-medicate with alcohol or other drugs as an avoidance coping strategy directed to reduction of symptoms of generalized autonomic hyperarousal, anxiety, fear and phobias subsequent to trauma (Bursztajn et al.; Jacobsen, Southwick, & Kosten, 2001; Shalev, 2001), this was not prevalent in the HH group in the present study. It may be that participants in this study abuse alcohol less frequently than those of other studies, particularly considering that alcohol was not a contributing factor in the MVC events in this study. However, as compared to the non-PTSD group, the PTSD group was significantly more likely to use substance abuse to cope at six weeks post-MVC. The latter finding is consistent with research that shows that PTSD develops first and alcohol or drug abuse arises secondarily (Bursztajn et al.). These findings support the hypotheses that high hyperarousal levels would be related to choice of coping strategies and perceived stress level. The hypothesis that perceived coping effectiveness would be related to hyperarousal level was not supported.

The HH group had higher perceived stress and higher perceived pain levels at session 1, but not at session 2. At both sessions, HH was associated with higher total coping scores. HH at session 1 was associated with coping by denial, disengagement, and self-blame. HH at session 2 was associated with coping by self-distraction, active coping, venting, self-blame and use of instrumental and emotional support (Table 5.3).

The findings regarding coping when subjects were grouped by hyperarousal level are similar to when they were grouped by ASD or non-ASD at session 1 (Tables 5.1 and 5.3). The only difference is that the HH group used more disengagement strategies whereas the ASD group used more self-distraction. Similarities between these groups in more frequent use of denial, self-blame, and overall coping effort is not surprising

because many of the same subjects comprised both groups. Also, hyperarousal is a symptom cluster for ASD.

Peritraumatic dissociation was associated with high hyperarousal levels at two and six weeks post-trauma and with PTSD. Although several studies, including the present study, found that peritraumatic dissociative reactions are associated with increased risk for PTSD and increased PTSD symptom severity (Classen, Koopman, Hales, & Spiegel, 1998; Grieger et al., 2000; Yehuda & Wong, 2000), others found that dissociation does not add predictive power for the development of PTSD to the overall diagnosis of ASD (Harvey & Bryant, 1999c; Marshall & Schell, 2002). Many dissociative symptoms listed for ASD can be found under other criteria in PTSD, producing controversy about whether ASD is actually early PTSD (Koopman, Classen, & Spiegel, 1994; Marshall, Spitzer, & Liebowitz, 1999). Others suggested that dissociation is a coping strategy that helps to protect psychological integrity (Harvey & Bryant, 1999c; Medina, Mejia, Schell, Dawson, & Margolon, 1999). Despite this, the use of dissociation as a coping strategy has been associated with increased risk of PTSD (Classen et al.). One reason for this may be that dissociation impairs the emotional processing that is needed for effective coping (Rachman, 2001). Consistent with this premise, MVC survivors in the present study who met dissociation criteria used disengagement, denial and self-distraction coping strategies significantly more frequently two weeks post-MVC.

The incidence of comorbid depression in the PTSD group in the present study (12.5%; $n = 3$) is lower than has been reported elsewhere (Khouzam & Donnelly, 2001: 20%; Shalev et al., 1998: 40%). The finding that the incidence of depression was not higher in females also contrasts with other studies (Maes et al., 2000). However, the sample with depression is too small for valid comparisons.

The impact of traumatic events may be reflected in changes in cortisol levels. Cortisol levels rise in response to an acute stressor in healthy individuals without PTSD (Yehuda, 2001). However, cortisol responses reported in the present study and in other studies of people in the immediate aftermath of trauma suggest that cortisol levels can be low in response to an extremely traumatic experience, before criteria for a diagnosis of PTSD are met (Bourne, Rose, & Mason, 1968; de Kloet, Vermetten, Geuze, Wiegant, & Westenberg, In press). In the present study, LH and HH groups showed no differences in awakening baseline salivary cortisol levels. However, in the early and later post-MVC periods, the HH group showed lower daytime cortisol responses that are consistent with hypocortisolism that has been reported in PTSD (Olff, Langeland, Draijer, & Gersons, 2007; Yehuda, 2001, 2002). Also, at both sessions, all subjects in the LH group who had low baseline and high daytime cortisol levels developed neither ASD nor PTSD. Together, these findings suggest that acute cortisol responses to recent trauma may be different in individuals who subsequently develop PTSD from those who do not. These differences may indicate that HPA axis adaptation begins early, that preexisting HPA axis changes were already present at the time of the trauma, or that ASD is early emerging PTSD. Alternatively, other factors that influence cortisol responses in traumatic events have yet to be identified. Prospective studies that measure cortisol in the immediate aftermath of trauma and periodically thereafter are needed to more fully understand the acute HPA axis response to highly traumatic events.

In the present study, expected associations between the SAM and HPA systems and the SAM system and PTSD were not found. As measures of autonomic arousal, it was hypothesized that startle reactivity and heart rate would be positively correlated and that both would be elevated in the HH group. It was also hypothesized that the PTSD group would show exaggerated startle responses consistent with DSM-IV PTSD criteria

and with the theoretical association of PTSD with progressive neuronal sensitization. Instead, lower mean startle amplitudes were found in the HH and PTSD groups in response to startle stimuli, when presented alone or following a prepulse, that are suggestive of suppression of the startle reflex. Although several studies, including that of Shalev, Peri, Brandes, Freedman, Orr, and Pitman (2000), report sensitization of physiological reactivity (increased startle and heart rate) as PTSD progresses, other studies suggest that PTSD may be related to suppression rather than sensitization of physiological reactivity (Blanchard, Hickling, Galovski, & Veazey, 2002; Casada & Roache, 2006; Griffin, Resick, & Mechanic, 1997; Medina et al.,1999). Others found that early post-trauma heart rate is weakly or non-predictive of later PTSD (Kraemer, Moergeli, Roth, Hepp, & Schnyder, In press). Together, these results indicate that physiological suppression may occur in PTSD regardless of whether the traumatic event occurs only once and is limited in duration, such as MVCs, or is one of repeated victimization or prolonged duration. The neuroendocrine basis for sensitization versus suppression of physiological reactivity has yet to be clarified.

The pattern of progressively decreasing startle amplitude across the four blocks of trials with a significant decrease in amplitude between the first and last blocks that is considered evidence of normal habituation (Falls, 2000) was not observed in ASD, non-ASD, PTSD, or non-PTSD groups. Although startle amplitude decreased between the first and last blocks of startle trials in both the ASD and PTSD groups, providing evidence that some habituation occurred, habituation was not significant. This process of habituation may be impaired in PTSD (Shalev et al., 2000). Shalev et al. found that subjects with PTSD 4 weeks following acute traumatic events of various types required more stimulus trials to habituate. Controlling for effects of prior trauma and other extraneous variables did not yield significant differences in habituation. Thus, the

secondary hypothesis that MVC survivors with ASD and no history of prior trauma would show normal startle habituation was not supported. Also, the hypothesis that the PTSD group would take longer than the non-PTSD group to habituate was not supported. One reason for lack of support for these hypotheses may be the marked degree of inter-subject variability in startle that is characteristic of this sample of MVC survivors.

Neither the LH nor HH groups showed significant habituation. Diminished habituation was observed in both LH and HH groups and not specific to the HH group as hypothesized. Mean startle amplitude increased in the LH group and decreased in the HH group from session 1 to session 2. This contrasts with the hypothesis that higher levels of hyperarousal would be associated with greater startle amplitude. Mean startle amplitude differed significantly between LH and HH groups in blocks 2 and 4 at each session. No significant effects of hyperarousal on PPI (sensorimotor gating) were found.

The startle results in this study that contrast with hypotheses and other studies can not be explained by startle assessment equipment or parameters, since a separate study in healthy women was conducted concurrently using the same equipment and startle paradigm; that study showed significant habituation across blocks (see Chapter 4). Although the present study assessed habituation to pulse alone trials, these trials were intermixed with prepulse trials. Other MVC studies have not assessed PPI. It may be that there are important trauma-related differences in the ways in which MVC survivors respond to intermixed stimuli. Pacak (2000) found preliminary evidence of distinctive neuroendocrine stress response patterns to different stressors in animal studies that suggest there may be stressor-specific differences in the activation of the HPA and SAM systems. Waters and Ornitz (2005) found that there may be some degree of independence in neuronal input to the levator palpebrae and orbicularis oculi muscles, both of which are involved in the eyeblink reflex and influence eyeblink as measured by

the equipment used in the present study. Other MVC studies commonly use electrodes that measure only the orbicularis oculi muscle reflex.

In contrast to previous evidence that cortisol may modulate the startle reflex (Buchanan et al., 2001), an association between startle reactivity and cortisol was not found. These findings underscore the complexity of the interactivity of the SAM and HPA neuroendocrine pathways and the need for further research to better understand how these pathways may be differentially affected by various types of traumatic experiences.

The life events inventory of the CAPS-I does not specifically ask about early childhood traumatic events, although the questions around physical or sexual assault can include both childhood and adult experiences. However, emotional abuse in childhood is not addressed at all in the inventory, which is a limitation of its use. Research has shown that chronic physical and/or emotional neglect or abuse during childhood can lead to permanent changes in neurohormonal regulation of the stress response and changes in brain development (Chalk, Gibbons, & Scarupa, 2002; Schore, 2001;Teichera, Andersena, Polcarib, Andersona, Navaltaa, & Kima, 2003). Children may respond to early childhood traumas with a hyperarousal or dissociative response or both (Perry 2000, 2002). Thus, findings of this study regarding dissociation, hyperarousal and neurohormonal reactivity could potentially have been influenced by any childhood emotional neglect or abuse experienced by the study population.

The between-subject variability in some of the neurophysiological parameters of this study limits reliable interpretation of some physiological data. For some variables, bar charts showed large differences in means whereas statistical analyses showed a lack of significance. Examples include the relationships between ASD and startle amplitude in Figure 5.3, between PTSD and PPI in Figure 5.8, and between hyperarousal level and baseline cortisol in Figure 5.9. The large standard deviations for

these variables decrease ability to achieve statistical significance. A large number of diverse factors – such as age, gender, type of trauma, medications, and comorbid health problems – may influence neurobiological and behavioral responses to traumatic stress and contribute to variability. To adjust for some of this inherent variability, researchers may need to recruit large samples or be highly selective about sample characteristics and nature of the traumatic event.

Conclusion

MVCs are among the most frequent traumatic events culminating in PTSD. Traumatic events can shatter individuals' views of the world as a safe place and can cause considerable distress long after the event. Potentially, they can exert long-lasting effects on psychological and physical health. Attempting to attenuate these adverse effects is a critical health issue. Yet, relatively few prospective studies in MVC survivors have included physiological measures of stress. This study has contributed to the development of knowledge regarding the early emergence of trauma symptoms, psychological and biological correlates, and coping in MVC survivors. Studies such as this that utilize integrative, multidimensional research approaches may lead to increased psychobiological understanding of traumatic stress symptomatology, earlier identification of those at risk for developing PTSD, and eventually lead to more effective attenuative and restorative interventions for people with ASD and PTSD.

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CHAPTER SIX

Postscript

In this postscript, I share some of my reflections on my research work and revisit questions that arose during the conduct of my research.

The results of my doctoral work support the value of using an integrative, multidimensional approach in stress research. My study of early emerging psychological and contextual variables that contribute to the development of ASD and PTSD following a motor vehicle crash and exploration of their linkages to neurobiological tests of autonomic arousal and HPA axis activity is a strong example of the value of such an approach. Findings regarding acoustic startle, heart rate and salivary cortisol patterns would have been difficult to interpret without analyzing them in the context of traumatic stress symptoms and coping patterns. For example, hyperarousal was assessed using psychological and physiological measures. As measured psychologically (by the IES) and consistent with my primary hypothesis, high hyperarousal levels two weeks and six-weeks post-MVC were strongly associated with PTSD. It was hypothesized that startle reactivity and heart rate, as physiological measures of hyperarousal, would be positively correlated and that both would be elevated in the HH (IES) group. Instead, no differences were found in heart rate and lower mean startle amplitudes were found in the HH and PTSD groups in response to startle stimuli. Had physiological measures been used without psychological measures, I may have been concluded erroneously that there were no differences in hyperarousal in this sample of MVC survivors. The standard deviations in the startle data were very large and showed a high degree of inter-subject variability that underscores the complexity of traumatic stress responses and the importance of integrative, multidimensional approaches to stress research.

During the course of my research work, questions emerged that required answers. One of these concerned whether the blood in blood-tinged saliva samples from micro-injury of the oral cavity was sufficient to influence salivary cortisol levels. A few blood-tinged saliva samples were subsequently assayed for transferrin as a means of quantifying blood leakage. At the time of preliminary testing in our lab, a study was published that showed that the presence of transferrin, thus blood contamination, did not significantly influence salivary cortisol levels (Kivlighan, Granger, Schwartz, Nelson, Curran, & Shirtcliff, 2004). Since results of tests in our lab were consistent with the latter article, further testing was deemed unnecessary.

A second question arouse as to whether there may be differences in startle reactivity between the women in my MVC study and the healthy women in my Menstrual Phase (MP) study. Startle amplitude and habituation were compared between follicular phase women in the MP study (n = 17) and females of childbearing age at two weeks post-MVC in the MVC study (n = 11). Six of the females in the MVC group were on oral contraceptives. Females in these groups did not differ significantly in age or other extraneous variables. Although females in the MVC group rated their perceived stress levels significantly higher than did those in the MP group ($F = 10.71$, $df = 1, 27$, $p = 0.003$), there was no significant correlation between perceived stress and startle amplitude; this lack of a correlation is consistent with findings in my MP Study (Chapter 4). Females in the MP and MVC groups did not differ in startle amplitude in response to pulse alone stimuli, either within or between groups in any of the four blocks of trials. However, mean amplitude in the first trial block was higher in the MP group and approached significance ($F = 3.18$, $df = 1, 27$, $p = 0.086$). A normal habituation pattern was observed in both MP and MVC women (see Figure 6.1). Habituation, using difference scores, was significantly greater in the MP group ($F = 4.20$, $df = 1, 27$,

$p = 0.051$). These findings of higher perceived stress and diminished habituation in females post-MVC provide evidence that trauma may disrupt adaptive neurohormonal activity in women.

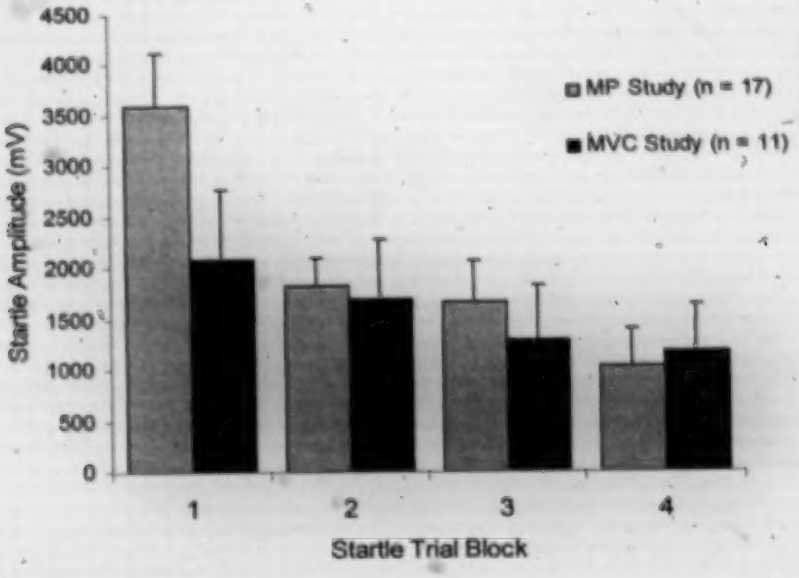

Figure 6.1. Startle amplitude and habituation in MP females as compared to MVC females.

An integrative approach to research has implications for nursing. As discussed in Chapter Two, nursing has shifted its focus from an empiricist paradigm toward an interpretivist human science paradigm, with its emphasis on holistic understanding of the experiences of unique human beings. While there are many positive aspects to this movement, a holistic approach to care and research must necessarily encompass the biological as well as the psychological and social aspects of being human. It is critical that as we seek to understand cognitive aspects of traumatic experiences we also seek to understand more fully their biological basis and implications for health. Through such an integrative approach, nurses may generate knowledge that ultimately enhances professional competence and improves the quality and safety of client care.

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Appendix A

UNIVERSITY OF ALBERTA

INFORMATION ABOUT THE STUDY

Title of Project:

Physiological and emotional stress responses and coping in people who have experienced motor vehicle crashes

Principal Investigator: Shirley L. King, RN, MN, Doctoral Candidate

Dissertation Supervisor: Dr. Kathy Hegadoren, RN, PhD

Purpose of the Study: Changes in stress hormone systems in the body can occur after experiencing a stressful event, such as a motor vehicle crash. Changes in stress hormones can influence our emotions, heart rates, how easily we startle, how well we sleep, and other symptoms. Changes in stress hormones following a stressful event may be greater and longer lasting and have a greater impact on health in some people than in others. Coping and other factors may influence stress responses. This study will compare stress symptoms, ways of coping, cortisol (a major stress hormone) and startle responses in people soon after being involved in a motor vehicle crash and then again about four weeks later. This study will help us to learn more about individual differences in stress responses and how they change over time. It will also help us to understand why some people may be more likely than others to have changes in stress responses that could affect health.

Procedures: The study is divided into two parts that will take place on two different days. The total time required for the first part of the study will be about 2 hours, depending mostly on how much time is used to answer questions. You will not be rushed and may set the pace. The time required for the second part of the study will be approximately 1 hour and 30 minutes. A qualified member of the research team will be present for all parts of the study at the Research Unit.

First Day (Part I):

During the first part of the study, your response to sounds that may startle you will be assessed. Healthy people startle in response to sudden or unexpected sights and sounds during the course of a normal day. Movies commonly combine sounds and images to periodically startle viewers. Startle is a normal reflex and an important part of the stress response, even though it may be mild and involve little more than an eyeblink. Startle will be assessed in a private room that is a part of the Research Unit. (The equipment that will be used will be shown and explained before you agree to take part in the study). Headphones will be placed over your ears. You will hear low continuous tones that sound much like static on a radio with poor reception. Occasionally, you will hear louder tones that last less than a second at levels that will not hurt your hearing, but may cause you to startle mildly and blink your eyes. Often the louder sounds will be so brief that they are barely perceptible to you. At the same time, you will wear eyeglasses that are fitted with small devices that use a tiny beam of

light to record eyeblinks will be worn. You will be able to see quite well while the glasses are on, but some of your vision will be blocked as much as it would if you held a finger about 2 cm away from and directly in front of each eye. You are unlikely to notice the tiny light beams which will record when your eyes blink. You will have a few minutes to try out the eyeglasses and headphones, ask questions, and decide if you still wish to participate before any startle assessment is done.

At the same time as startle is measured, your heart rate will be counted. A small fingertip device is used to count pulse rate.

You will be asked to provide a saliva (spit) sample that will be used to measure cortisol (a stress hormone) just before and after each startle assessment. You will be given a small plastic tube with a sterile cotton roll inside. To provide a sample, you are asked to remove the cotton roll, chew it for one full minute, and then replace it in the tube. You will be asked to collect another specimen at home the morning after this appointment at the Research Unit, then post it in the prepaid addressed envelop that is provided. You will be asked to collect another sample at home the morning before your second appointment at the Research Unit and to bring it with you to your appointment. You will be given written instructions and the small plastic tubes with a sterile cotton rolls inside to take home.

The actual heart rate and startle assessment will take about 25 minutes. When it is finished, you will be given an opportunity to talk about the experience and ask questions.

Following the startle assessment, you will be asked questions about feelings you may have, how stressed you feel, the ways you cope and your medical history. If you choose to take part you do not have to answer every single question, or tell us anything that you choose to keep private. There are no right, wrong, good, or bad answers to any of the questions.

If you are willing to return to finish the second part of the study, an appointment can be made at this time.

Second Day (Part 2): The second part involves returning to the Research Unit about four weeks after your accident for a second interview and startle and heart rate assessment. You will be asked how stressed you feel, stress symptoms, and how you are coping. The startle, saliva sample, and heart rate procedure will be the same as that used during the first session.

Other information obtained:

Because the severity of injuries that result from an accident may influence stress responses, the severity is commonly rated by physicians in the emergency department. If you consent to take part in the study, this rating and a description of the accident will be obtained from the emergency department and used in this study. This information will remain confidential and no other information will be obtained from your record.

Compensation: You will receive $5.00 for parking/transportation expenses on each of the days that you come to the Research Unit.

Benefits: There is no specific benefit to you in taking part in this study, other than a sense of contribution to the scientific understanding of stress responses. This might lead to improved care for people who suffer with health problems from too much stress.

Risks: A risk of taking part in this study is that you might feel anxious from the use of equipment which is not familiar to you. The startle assessment will feel a lot like wearing sunglasses and headphones at the same time. You might also feel anxious about hearing sounds that startle you and make you blink. You might also feel excitement and adventure at trying this kind of assessment. You can stop the startle assessment at any time without explanation by telling the investigator of this decision. If you wish to talk about the experience, you will be welcome to do so.

After chewing on the cotton dental role for one minute to provide a saliva sample, your mouth may feel dry for a minute or two.

Another risk is that you might feel anxious from being asked questions about your emotions and ways you are coping since your accident. You do not have to answer every question and may stop the interview at any time without explanation. Referral to mental health services will be provided should these services be needed. You will also be provided printed information about mental health services and how to contact them, should you wish to do so at any time. You might also benefit from the opportunity to talk about your emotions and coping following the crash.

Confidentiality: All information will be held confidential, except when professional codes of ethics or the law requires reporting. All consent forms will be stored separately in a locked cabinet. Real names will not be included on any other records. In place of real names, each participant will be given a code number.

The information you provide will be kept for at least five years after the study is done. The information will be kept in a locked file cabinet in a secure area. Your name or any other identifying information will not be attached to the information you provide. Your name will also never be used in any presentations or publications of the study results.

The information gathered for this study may be looked at again in the future to help us answer other study questions. If so, the ethics board will first review the study to ensure the information is used ethically.

Right to withdraw: You are free to withdraw from the study at any time without explanation. If you have further concerns about any aspect of this study, you may contact the Office of the Associate Dean (Research) in the Faculty of Nursing at 492-6764. This office has no affiliation with the researchers involved in the study.

Please contact either of the individuals identified below if you have questions or concerns. If we are unavailable at the time of your call, please leave a time when we can return your call or you can call us back if you prefer.

Shirley King, tel: 492-3835 or 492-9042

Dr. Kathy Hegadoren, tel: 492-4591, or pager: 401-0368

Appendix B

Consent to Participate in a Research Study

Part 1 (to be completed by the Principal Investigator):

Title of Project: Physiological and emotional stress responses and coping in people who have experienced motor vehicle crashes

Principal Investigator: Shirley L. King, RN, MN, Doctoral Candidate
Faculty of Nursing, University of Alberta
Phone: 492-9042

Research Supervisor: Dr. Kathy Hegadoren, RN, PhD
Faculty of Nursing, University of Alberta
Phone: 492-4591

****Please read the attached 'Information about the Study' carefully before signing this**

Part 2 (to be completed by the research subject):

Do you understand that you have been asked to be in a research study?	☐ Yes ☐ No
Have you read and received a copy of the attached Information Sheet?	☐ Yes ☐ No
Do you understand the benefits and risks involved in taking part in this research study?	☐ Yes ☐ No
Have you had an opportunity to ask questions and discuss this study?	☐ Yes ☐ No
Do you understand that you are free to refuse to participate or withdraw from the study at any time? You do not have to give a reason and it will not affect your care.	☐ Yes ☐ No
Has the issue of confidentiality been explained to you? Do you understand who will have access to your records?	☐ Yes ☐ No

This study was explained to me by: _____

I agree to take part in this study.

_____	_____	_____
Signature of Research Participant	Date	Witness
_____		_____
Printed Name		Printed Name

I believe that the person signing this form understands what is involved in the study and voluntarily agrees to participate.

_____	_____
Signature of Investigator or Designee	Date

Part 3 (to be completed by the research subject):

If you are willing, a member of the research team would like to contact you about two days before your next appointment at the Research Unit to remind you about cortisol sample collection and the time of your appointment. You will be welcome to ask any questions that you may have about the study at that time.

I agree that a member of the research team may contact me about two days before my appointments at the Research Unit? ☐ Yes ☐ No

If 'Yes', please complete the following questions:

I may be contacted by phone:

☐ Yes ☐ No

The times that I may be called are:

☐ anytime; ☐ in the morning; ☐ in the afternoon; ☐ in the evening; ☐ never.

Telephone number at which I may be contacted:

Day ..... Evening .....

A message may be left:

On my answering machine ☐ Yes ☐ No
With anyone who answers ☐ Yes ☐ No
Do not leave a message ☐ Yes ☐ No

I may be contacted by email:

☐ Yes ☐ No

If 'Yes', email address: .....

_____ _____

Signature of Research Participant Date

Appendix C

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Dear Shirley,

Many thanks for your email regarding your paper 'An integrative science approach: Value added in stress research' that appeared in Nursing & Health Science, 8(2).

As long as the paper is clearly and correctly referenced as having been published in NHS (as you suggested in your email it would be), there is no problem at all in including your paper in your final submission.

Best of luck with your dissertation.

Kind regards,

Lindsey

(Miss) Lindsey Mathews

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